

Treatment of Obstructive Airway Disease With a Cysteine Donor Protein Supplement

A Case Report *

Bryce Lothian, MD; Vijaylaxmi Grey, Ph.D.; R. John Kimoff, MD and Larry C. Lands, MD, Ph.D.

From the Department of Pediatrics (Drs. Lothian and Grey), Department of Biochemistry (Dr. Grey), and Division of Respiratory Medicine (Dr. Lands), McGill University Health Centre Montreal Children's Hospital, Montreal, Quebec, Canada; and Division of Respiratory Medicine (Dr. Kimoff), McGill University Health Centre-Royal Victoria Hospital, Montreal, Quebec, Canada.

Correspondence to: Larry C. Lands, MD, Ph.D., Assistant Director, Respiratory Medicine, The Montreal Children's Hospital, Room D-380, 2300 Tupper St, Montreal, Quebec, Canada H3H 1P3

Abstract

Oxidant/antioxidant imbalance can occur in obstructive airways disease as a result of ongoing inflammation - Glutathione (GSH) plays a major role in pulmonary antioxidant protection. As an alternative or complement to anti-inflammatory therapy, augmenting antioxidant protection could diminish the effects of inflammation. We describe a case of a patient who had obstructive lung disease responsive to corticosteroids, and low whole blood GSH levels. After 1 month of supplementation with a whey-based oral supplement designed to provide GSH precursors, whole blood GSH levels and pulmonary function increased significantly and dramatically. The potential for such supplementation in pulmonary inflammatory conditions deserves further study.

Key Words: glutathione, inflammation, oxidative stress, and supplementation

Introduction

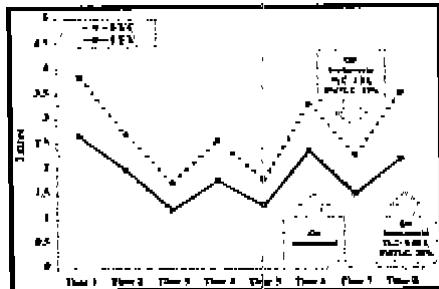
Evidence of oxidant/antioxidant imbalance has been demonstrated in obstructive airway disease.¹⁻² Continued lung inflammation with the mobilization and activation of neutrophils, macrophages, and eosinophils and their release of free oxygen radicals and other reactive oxygen species (ROS) is a source of oxidative stress. In addition to the

direct effects of such ROS on Cell membranes, DNA, and proteins, breakdown products act as signals perpetuating the inflammatory cascade. Glutathione (GSH) and the GSH system play a key role in protecting against the effects of ROS.^{3,4} Modulation of the oxidant/antioxidant status in obstructive airway disease primarily aimed at enhancement of the GSH system, has been limited by difficulties in delivery of an effective substrate.^{2,3,5} We describe the response to an oral, whey-based supplement designed to supply GSH precursors.

Case Report

A 40-year-old woman of North African origin was followed by the pulmonology service at a tertiary care hospital in 1997. Her medical history was significant for Hodgkin's lymphoma, diagnosed 27 years earlier and treated with radiation and chemotherapy. She had a 25-pack-year smoking history and had quit smoking in 1994. In 1995, she received a diagnosis of mild valvular heart disease (aortic and mitral regurgitation). At that time (time 1), pulmonary function tests (PFTs) suggested mild airflow obstruction (Fig 1); bronchodilators were not prescribed. In 1997 (time 2), she was admitted to hospital with a virally induced exacerbation at obstructive lung disease as well as mild heart failure. She improved with diuretics, bronchodilators (salbutamol and ipratropium bromide), and oral prednisone, 20mg/d. She was discharged taking a tapering course of prednisone.

Figure 1. Tracking of the pulmonary function values over time.



TLC = total lung capacity;
RV = residual volume.

When seen in follow-up 1 week later (time 3), she had suffered an exacerbation of her respiratory symptoms (shortness of breath, wheeze, chest tightness, and excessive mucus production) coincident with cessation of prednisone. Prednisone was prescribed again.

She returned 2 weeks later (time 4) with significant symptomatic improvement while still taking systemic corticosteroids and regular bronchodilators (salbutamol metered-dose inhaler and ipratropium bromide metered-dose inhaler qid). An attempt was made to discontinue prednisone. When the patient was seen 1 month later (time 5), her symptoms had returned. At that time, review of her history revealed no environmental insult that could account for her deterioration. Additionally, serum IgE was 52 kU/L (laboratory control, 0 to 100 kU/L), and both allergen skin testing and Aspergillus precipitins testing were negative. A further course of oral prednisone was prescribed (40 mg/d initially; tapering over 1 month).

Four months later (time 6), the patient returned to clinic independently, having begun taking HMS90 (Immunocal; Immunotec Research Ltd.; Vaudreuil, Quebec, Canada) a whey-based protein supplement (10 g bid), 1 month before. She had heard that the product could be helpful in inflammatory conditions, and had started taking the product of her own accord. She reported a remarkable improvement in her respiratory status and

had discontinued all inhalers and steroids, and was not taking any other supplements, medications, or over-the-counter therapies. She was asked to discontinue the Immunocal, and within 3 months her symptoms returned. PFTs were performed at this time (time 7). She then restarted Immunocal of her own accord and 1 month later (time 8), PFTs were again assessed. Additionally, whole blood GSH levels were measured before and 1 month after therapy was reinitiated, using a modification of the method previously described^{6,7}. Again, a remarkable improvement in both symptoms and PFTS was noted (E)1. In addition the total lung capacity increased from 3.91 L at time 7 to 5.00 L at time 8, and the residual volume/total lung capacity ratio fell from 33 to 28%. Her whole blood GSH levels increased from 235 to 457 $\mu\text{mol/L}$ (laboratory control, 589.2 plus or minus 112.6 $\mu\text{mol/L}$; n = 10). It should be further remarked that the last two PFTs performed showed reversibility of the obstructive airway disease (change in FEV₁, 48% at time 7 to 15% at time 8), whereas no prior PFT had shown reversibility. The patient continues to take HMS90 (Immunocal) and no other respiratory medications, without return of her symptoms.

Discussion

The patient suffered from a worsening of her previously diagnosed obstructive airway disease. The relative contributions of smoking, asthma, and cancer therapy to her baseline lung disease are unclear, as was the cause of her deterioration. She required multiple courses of systemic corticosteroids to maintain lung function. Symptomatic improvement correlating with pulmonary-function coincided with her initiation of HMS90. More significantly, pulmonary function worsened with withdrawal of HMS90 (Immunocal) and improved with re-introduction. Her final respiratory status is objectively and subjectively better than at any time in the previous 4 years.

HMS90 (Immunocal) is a bovine whey-protein concentrate purified by ultrafiltration and low-temperature pasteurization of milk. The undenatured whey protein is rich in cystine (the oxidized form of cysteine) and glutamylcystine which are precursors of GSH synthesis. The tripeptide GSH (glycyl- γ -glutamylcysteine) is synthesized in the cell in two steps. The first step, the synthesis of glutamylcystiene, is limited by the availability of intracellular cysteine⁴. As well, γ -glutamylcystiene, as a γ -glutamyl amino acid, can easily be transported into the cell where it combines with glycine in the second step of GSH synthesis⁸. Cells cannot take up extracellular GSH³.

In the patient described, whole blood GSH levels were significantly increased (94%) following regular intake of HMS90 (Immunocal). This is much higher than the reported intraindividual variation in whole blood GSH values (7.8 to 15.8%)⁹. In order to avoid any possible influence of the timing of sampling on GSH levels and pulmonary function, the patient was tested between 10:00AM and 11:00AM on each visit. Animal studies of GSH metabolism have demonstrated that whole blood GSH is reflective, temporally and quantitatively, of lymphocyte and tissue GSH levels. Although no direct markers of oxidant /antioxidant status or inflammation were measured in the patient described, the observed clinical effect is coincident with augmented GSH levels.

Several specific abnormalities, or inadequacies, of the GSH antioxidant system have been identified in reversible obstructive airway disease. GSH itself is present in high concentrations in the lung epithelial lining fluid (ELF), where it may act to directly reduce

ROS^{10,11}. Clinically stable asthmatics have higher ELF GSH than symptomatic asthmatics⁵, while experimental models of oxidative stress show an increase in ELF GSH with oxidative stress¹⁰. Upregulation of antioxidant defenses, although not in proportion to oxidative stress¹ is hypothesized to account for the increased BAL fluid GSH levels observed in both these studies and other pulmonary conditions (the) are attributed, in part, to excessive oxidative stress¹⁰, GSH is also a substrate for the decomposition of a large number of ROSs (including hydrogen and other peroxidases)⁴. Studies have shown decreased peripheral blood GSH-Px activity in asthmatic patients¹. Finally, it has been recently demonstrated in a murine model that GSH levels in the antigen presenting cell affect the differentiation of the T-helper cell Th1/Th2 cytokine response¹².

Improvement in GSH status could result in augmented lung function through several mechanisms. Within lung epithelial cells, augmented GSH may block the activation of nuclear factor α B by tumor necrosis factor- α ,^{13,14,15} and so limit the production and release of pro-inflammatory cytokines. Augmented intracellular GSH may reduce the need to recycle GSH from the lung lining fluid, and thus maintain extracellular levels¹⁶. Alternatively, increased intracellular GSH levels may lead to extracellular transport to buttress lung lining levels. In the lung lining fluid, augmented GSH may prevent oxidative damage to antiproteases^{17,18}. Improvement in skeletal muscle function due to augmented GSH stores⁷ may also partially account for our results, as the baseline FEV1/FVC ratio did not change between times 7 and 8 (66% and 62%, respectively).

The ELF GSH pool has been the target of direct administration of nebulized GSH, although success has been limited by GSH-induced bronchospasm⁵. Trials of systemic N-acetyl cysteine, acting as both a cysteine donor and an ROS scavenger, for the treatment of chronic obstructive airway disease have met with limited success, because of N-acetyl cysteine toxicity and limited clinical effect^{2,19}.

The relationship between whole blood GSH, lung epithelial cell GSH levels, ELF GSH, and peripheral blood GSH-Px activity is poorly defined. There are several possible mechanisms by which GSH could improve obstructive airway disease, either via immunologic modulation or by improving antioxidant defenses. More work needs to be done to further define the specific abnormalities of antioxidant function, as well as the relative contribution of such abnormalities to the pathophysiology observed in obstructive airway disease. Nevertheless, the modulation of GSH and antioxidant defenses in obstructive airway disease (and many other diseases) represents an intriguing potential modality for anti-inflammatory therapy.

Footnotes

Abbreviations:

ELF = epithelial lining fluid

GSH = glutathione

GSH-PX = glutathione peroxidase

PFT = pulmonary function test

ROS = reactive oxygen species

L.C. Lands and H.J. Kimoff are clinical investigators with the Fonds de la Recherche en Santé du Québec.

Received for publication June 21,1999-Accepted for publication September14, 1999.

References

1. Powell. CV, Nashl A,A, Powers, HJ, et al (1994) Antioxidant status in asthma. *Pediatr Pulmonol* 18,34-38 (Medline)
2. Repine, JE, Bast, A, Lankhorst, I(1997) Oxidative stress in chronic obstructive pulmonary disease: Oxidative Stress Study Group. *Am J Respir Crit Care Med* 156,341-357 (Full Text)
3. Anderson, ME (1997) Glutathione and glutathione delivery compounds. *Adv Pharmacol* 38,65-78(Medline)
4. Meister, A (1989) Metabolism and function of glutathione. Dolphin, D Poulsen, H Avramovic, O eds *Glutathione: chemical, biochemical and medical aspects* 367-474 John Wiley and Sons New York, NY.
5. Marrades, RM, Hoca, J, Barbera, JA, et al (1997) Nebulized glutathione induces bronchoconstriction in patients with mild asthma. *Am J Respir Crit Care Med* 156,425-430 (Abstract/Full Text)
6. Lands, LC, Grey, VL, Smountas, AA1 et al (1999) Lymphocyte glutathione levels in children with cystic fibrosis. *Chest* 116,201 -205 (Abstract/Full Text)
7. Lands, LC, Grey, VL, Smountas, AA (1999) Effect of supplementation with a cysteine donor on muscular performance. *J Appl Physiol* 87,1381-1 385 (Abstract/Full Text)
8. Anderson, ME, Meister, A (1983) Transport and direct utilization of gamma-glutamylcyst(e)ine for glutathione synthesis. *Proc Natl Acad Sci USA* 60,707-711 (Medline)
9. Richie, JP, Jr, Abraham, P, Leutzinger, Y (1996) Long-term stability of blood glutathione and cysteine in humans. *Clin Chem* 42,1100-1105 (Abstract)
10. Heffner, JE, Repine, JE (1989) Pulmonary strategies of antioxidant defense. *Am Rev Respir Dis* 140,531-547 (Medline)
11. Cantin, A, North, S, Hubbard, H, at a (1987) Normal epithelial Lining fluid contains high levels of glutathione. *J Appl Physiol* 63,152-157 (Medline)
12. Peterson, JD, Herzanberg, LA, Vasquer, K, et al (1998) Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. *Proc Natl Acad Sci USA* 95,3071 - 3076 (Medline)
13. Flohe, L, Brigelius-Flohe, R, Saliou, C, et al (1997) Redox regulation of NF-kappa B activation. *Free Radical Biol Med* 22,1115-1126 (Medline)
14. Palombella, VJ, Rando, OJ, Goldberg, AL, at al (1994) The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B *Cell* 78,773-785 (Medline)
15. Rahman, I, Macnee, W (1998) Role of transcription factors in inflammatory lung diseases. *Thorax* 53,601-612 (Full Text)
16. Hull, J, Venaart, P, Grimwood, K, et al (1997) Pulmonary oxidative stress response in young children with Cystic Fibrosis *Thorax* 52 557-560 (Abstract)

17. Hubbard, RC, Ogushi, F, Fells, GA, et al (1987) Oxidants spontaneously released by alveolar macrophages of cigarette smokers can inactivate the active site of alpha 1-antitrypsin, rendering it ineffective as an inhibitor of neutrophil elastase. J Clin Invest 80,1289-1295 (Medline)
18. Weiss, SJ (1989) Tissue destruction by neutrophils. N Engl J Med 320,365-376 (Medline)
19. Koch, SM, Leis, AA Stokic, DS, et al (1994) Side effects of intravenous N-acetylcysteine (abstract. Am J Respir Crit Care Med 149,A321)