Glutathione

A Radical Treatment for Cystic Fibrosis Lung Disease?

In this issue of CHEST (see page 308), Bishop and colleagues report encouraging results from a small, double-blind, placebo-controlled clinical trial of inhaled glutathione in patients with cystic fibrosis. The rationale for this therapy lies in previous data reporting that glutathione levels in the lung epithelial lining fluid of patients with cystic fibrosis are low, and that cystic fibrosis lung disease is associated with increased oxidative damage. In addition, more recent studies have also demonstrated that the cystic fibrosis transmembrane conductance regulator (CFTR), which is defective in cystic fibrosis, regulates a substantial portion of glutathione efflux into the epithelial lining fluid.

Glutathione is a ubiquitous thiol-containing tripeptide with a number of important biological functions (Fig 1). Glutathione is a unique peptide that is resistant to proteolysis due to a γ-glutamyl linkage to cysteine that requires a specific protease for degradation, γ-glutamyl transpeptidase. Glutathione is utilized by the body in a number of detoxification pathways. The detoxification pathways utilize the thiol group for xenobiotic metabolism (glutathione-S-transferases), a source of reducing power for detoxifying peroxides (glutathione peroxidases), and for protein repair (glutaredoxin). The oxidized glutathione disulfide (GSSH) is recycled by GSSH reductase. In addition, glutathione is a major determinant of tissue redox status, which is determined by the ratio of reduced to oxidized glutathione. Optimal extracellular redox environment is important in maintaining the structure and functions of plasma membrane proteins. Glutathione plays a dual role as a redox regulator and antioxidant.

The lung concentrates glutathione in its epithelial lining fluid; however, it is still not clear how or why the lung accomplishes this feat. It is also known that the levels of glutathione in the epithelial lining fluid are diminished in a number of lung disorders and environmental exposures including ARDS, idiopathic lung fibrosis, lung transplantation, HIV infection, alcohol abuse, asbestos, and cystic fibrosis. Studies in cystic fibrosis have partially illuminated mechanisms the lung uses to place glutathione into the epithelial lining fluid. This genetic disorder results in a defect in the CFTR protein that is an organic anion efflux channel related to the multidrug resistance protein family of adenosine triphosphate-binding cassette proteins. A series of in vitro studies have demonstrated that CFTR regulates glutathione efflux in epithelial cells and in artificial proteoliposomes. CFTR knock-out mice also have diminished glutathione levels in their lung epithelial lining fluids that is associated with increased lung oxidative stress. The pathophysiology of cystic fibrosis lung disease involves a continuous cycle of chronic Pseudomonas aeruginosa infection and inflammation that is thought to contribute to the progressive loss in lung function. A recent study has found that the lung adapts to oxidative stress mediated by P aeruginosa infection by effusing glutathione into the epithelial lining fluid, and this mechanism was defective in CFTR knock-out mice. A number of questions still remain to be answered dealing with lung glutathione efflux pathways, and include which factors stimulate glutathione efflux, the other transporter(s) that account for non-CFTR glutathione efflux, and better linkage between altered glutathione efflux pathways and lung pathophysiology.

There are now three small clinical trials that have examined the effects of inhaled glutathione in
patients with cystic fibrosis. Roum and colleagues\textsuperscript{16} were first to report the use of inhaled glutathione (600 mg bid for 3 days, approximately 17 mg/kg/d) in seven patients with cystic fibrosis. The glutathione therapy was well tolerated and produced a twofold increase in epithelial lining fluid glutathione levels. In addition, these investigators found diminished superoxide production from BAL cells after glutathione therapy. One concern raised by these studies was the rapid rise in oxidized glutathione levels in the treated patients with cystic fibrosis. The percentage of oxidized glutathione in untreated patients with cystic fibrosis was 46\%, and increased to 66\% after treatment. This increase in oxidized glutathione can adversely change the redox status of the epithelial lining fluid, and may explain why Griese and colleagues\textsuperscript{17} failed to see a change in the oxidative state of the epithelial lining fluid proteins and lipids in their inhaled glutathione study. The study by Griese and colleagues\textsuperscript{17} used a highly efficient inhalation delivery system (86\% of the emitted dose) to treat 17 patients with cystic fibrosis with two different regimens of glutathione (300 mg and 450 mg tid for 14 days, approximately 13 mg/kg/d and 20 mg/kg/d, respectively). Again, these regimens were well tolerated, increased epithelial lining fluid glutathione levels threefold to fourfold, and were associated with a significant improvement in the patient’s FEV\textsubscript{1}. However, a weakness of the two previous studies\textsuperscript{16,17} is that neither were placebo controlled. The Bishop study is the first double-blind, placebo-controlled trial reported for inhaled glutathione in 19 patients with cystic fibrosis. The study by Bishop et al used a higher glutathione dosage (66 mg/kg/d, divided into four inhalations, for 8 weeks). This regimen was also well tolerated and associated with an improvement in peak flow over placebo group, along with a trend toward improvement in a number of other clinical indicators. Given the small size of the trial and the mild airway dysfunction in the cystic fibrosis population studied, these results are encouraging that inhaled glutathione therapy could be beneficial. In summary, inhaled glutathione therapy was well tolerated and efficacious in improving a variety of clinical indicators in all three studies\textsuperscript{16–18} reported.

There is still lack of consensus regarding an appropriate glutathione regimen, and which primary and secondary indicators to be monitored. One view is to normalize glutathione levels in the epithelial lining fluid of patients with cystic fibrosis to normal. However, recent findings show that the epithelial lining fluid glutathione levels are elevated on infection with \textit{P. aeruginosa} and may argue for modulating glutathione levels according to infection status.\textsuperscript{15} It is also not clear whether the primary benefit of inhaled glutathione is mediated by acting as an antioxidant, since the study by Griese and colleagues\textsuperscript{17} failed to show decreases in levels of oxidized lipids and proteins in the epithelial lining fluid. However, one should note that these elevated levels of oxidized proteins and lipids in patients with cystic fibrosis likely changed slowly over the course of many years, and thus are unlikely to rapidly decrease with short-term therapy. Another potential approach is to stimulate glutathione efflux pathways to elevate endogenous glutathione in the epithelial lining fluid through non-CFTR–dependent pathways. This approach may avoid the accumulation of oxidized glutathione and disruption of the epithelial lining fluid redox status.

With three small clinical trials\textsuperscript{16–18} with positive findings now published, it seems clear that the next logical step is a large multicenter clinical trial. Several obstacles remain to be overcome. These include the cost of safety studies, agreement on dosages, primary indicators, and support from the pharmaceutical industry for an orphan indication. Given that a number of inflammatory lung diseases share a diminished level of glutathione in the epithelial lining fluid and excessive lung inflammatory responses, a glutathione therapeutic may have broader implications than cystic fibrosis. Glutathione may indeed be a radical approach to treat a number of inflammatory lung diseases.

\textbf{Brian J. Day, PhD}

\textit{Denver, CO}

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\textbf{Correspondence to:} Brian J. Day, PhD, Associate Professor of Medicine & Pharmaceutical Sciences, National Jewish Medical & Research Center, K715, 1400 Jackson St, Denver, CO, 80206; dayb@njc.org

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