Erythrocytic Glutathione in Cystic Fibrosis*
A Possible Marker of Pulmonary Dysfunction

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To evaluate the role of red blood cell (RBC) antioxidants as clinical markers of oxidative exposure, we measured RBC glutathione (GSH) concentrations in 32 adult patients with cystic fibrosis (CF), and 8 healthy age-matched control subjects. We chose patients with CF because this disease is characterized by severe bronchial inflammation and marked oxidant-antioxidant imbalance. Although the GSH concentration of the two study groups was not significantly different, the RBC GSH concentration of patients with CF had a greater variability (p=0.01) and was also inversely and significantly correlated to tests of pulmonary function (p<0.05). These data indicate a large and significant interindividual variability of erythrocytic antioxidants in patients with CF, with a compensatory, but probably inadequate, increase in patients with more severe respiratory deterioration. Red blood cell GSH concentration may thus provide a biologic marker for disease severity and a rationale for antioxidant manipulation in these patients.

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CF=cystic fibrosis; GSH=glutathione

Recent reports have indicated that red blood cell (RBC) antioxidants may function as somatic scavengers in many situations of oxidative stress to the respiratory system. In these circumstances, the RBC antioxidant activity (traditionally relegated to the reduction of hemoglobin-bound iron) would go well beyond the erythrocytic intracellular content, and would extend to plasma proteins, and other blood cells and surrounding tissues. Examples of this function are the capacity of RBC antioxidants to prevent in vitro vascular leakage and edema of isolated rat lungs exposed to hydrogen peroxide, decrease the injury of ischemic isolated hearts and, when insufflated into the trachea, dramatically improve the survival of rats exposed to 95 percent oxygen. Red blood cell antioxidants are also capable of undergoing adaptive changes upon exposure to an oxidative stress. For example, glutathione (GSH) concentrations are increased in RBCs of healthy smokers and patients with silicosis when compared with age-matched controls. Whether this increase may correlate with the degree of pulmonary dysfunction, and thus provide a clinical marker of functional severity, is not known.

Cystic fibrosis (CF) is a hereditary disorder characterized by progressive airways inflammation and unopposed elastolytic activity leading to early and severe deterioration of respiratory function. The erythrocytic GSH system of these patients has been variably reported as either increased or unchanged. We postulated that such differences might be explained by interindividual variability in the degree of oxidant-antioxidant imbalance suffered by these patients. If so, concentrations of GSH might correlate with the clinical severity of CF. To test this hypothesis, we measured RBC GSH concentrations in a group of adult patients with CF and correlated them with various clinical parameters of severity. Our results support our premise.

MATERIALS AND METHODS

Study Design

Thirty-two adult patients with CF and 8 age-matched controls represented the study population. The study was approved by the Institutional Review Board of the Medical College of Pennsylvania and informed consent was obtained from all participants prior to initiation of the investigational protocol.

Clinical Data

All controls were healthy, active, and nonsmokers. All patients with CF were ambulatory, active, and free from clinically symptomatic viral or bacterial respiratory infections. Each patient underwent a clinical interview to record respiratory symptoms and smoking habits, both current and past. White blood cell counts were measured and clinical severity scores were generated according to Taussig et al.12 These scores are computed by assigning various points for the presence of clinical, roentgenologic, or physiologic abnormalities. The total number of points a patient receives is then subtracted from 100 to obtain the final prognostic score. Mild dysfunction usually corresponds to scores between 85 and 100, while patients with a score of 50 or less rarely survive 3 years.

Pulmonary Function Tests

Spirometry was performed by means of a computer-assisted spirometer (Sensor-Medics 2450, Sensor-Medics Inc, Anaheim,
Calif). Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV\textsubscript{1}) were taken as the best of three satisfactory respiratory tracings. The FVC and FEV\textsubscript{1} percent values were referred to the Morris-Polgar predictive values.

**Measurement of Reduced GSH**

The RBC GSH Concentration was measured using a modification of the protocol reported by Beutler.\textsuperscript{15} Blood samples were collected via venipuncture from each participant. Six milliliters of blood was drawn into heparinized specimen (Vacutainer) tubes and samples were refrigerated at 5°C until processed. All samples were assayed within 24 h. Specimens were initially centrifuged (10 min, 3,000 rpm) and the supernatant consisting of plasma and buffy coat cells was decanted. The remaining RBCs were then washed three times in normal saline solution and again centrifuged using the same protocol. Three hundred to 600 µl of the packed RBCs were then hemolyzed by vigorous agitation for 1 min in a solution of 0.1 percent EDTA (pH 7.0) at a concentration of 15 to 30 percent. Then 400 µl of this hemolysate was removed for determination of hemoglobin concentration (Hgb) using a hemoximeter (OSM3, Radiometer, Copenhagen). An additional 400 µl of the hemolysate was mixed with an equal volume of 25 percent (w/v) glacial metaphosphoric acid, vortexed, and centrifuged at high speed for 1 min. Then 250 µl of the clear supernatant was added to 1.0 ml of phosphate buffer (0.5 M Na\textsubscript{2}HPO\textsubscript{4}) plus 250 µl of 5.5-dithiobis-2-nitrobenzoic acid at a pH of 6.8 and the absorbance of the entire solution was read spectrophotometrically at a wavelength of 412 nm.\textsuperscript{15} Standard curves were generated using identical conditions but defined concentrations of GSH. All measurements were performed in triplicate. The GSH content of each sample was then calculated using linear regression analysis and expressed as nanomoles per gram of Hgb.

**Expression and Analysis of Data**

Statistical analysis was performed on a PC microcomputer using a statistical package (SPSS, SPSS Inc, Chicago). Comparison of the GSH concentration in each subject was performed using the Student's t test. Analysis of GSH changes in the same subject was as a result of time was carried out by paired, two-tailed Student's t test. Correlation between GSH concentrations and patients' functional parameters (clinical severity score, white blood cell count, and pulmonary function tests) was examined by Pearson's product-moment correlation coefficient. Data dispersion was expressed as 1 SD and significance was set at a level of 95 percent or greater (p<0.05).

**RESULTS**

Figure 1 shows boxplots summarizing the distributions of GSH concentrations in patients with CF and healthy controls. Although patients with CF as an average had a higher GSH concentration, there were no significant differences in GSH concentrations between the two groups (2,269.9 ± 170.5 and 2,434.6 ± 539.6, respectively, p=0.15 by Student's t test). The GSH concentration of the CF group, however, was significantly more variable than the control group (p=0.01). By separating the 32 patients with CF into two subgroups on the basis of their FEV\textsubscript{1} percent predicted, there were significant differences between the control group and the two CF subgroups with mild or severe pulmonary dysfunction (FEV\textsubscript{1} percent predicted less than 50 percent, n=24, and FEV\textsubscript{1} percent predicted equal or greater than 50 percent, n=8; p=0.047 by analysis of variance). When correlations between RBC GSH concentrations and parameters of pulmonary function were carried out, there was a significant correlation for the FVC (Fig 2, r=0.35, p=0.035) and for the FEV\textsubscript{1} percent predicted (Fig 3, r=0.30, p=0.047), while there was a strong tendency toward significance for the FVC percent predicted and the FEV\textsubscript{1} (both p values = 0.08). There were no significant correlations between GSH concentration and clinical score (r=−0.12, p=0.257) or between GSH concentration and white blood cell count (r=0.17, p=0.19).

A small group of patients with CF had blood samples drawn at 6 and 12 weeks to study the possible effects of time on the RBC GSH concentration of the same subjects. All these patients remained in clinically stable conditions, and analysis of GSH concentration was never carried out during an acute exacerbation. There were no significant differences between baseline GSH concentration and GSH concentration measured at 6 weeks (2,214.8 ± 544.6 and 2,346.5 ± 620.1, respectively, p=0.56, n=14) or 12 weeks (2,566.1 ± 589.1 and 2,637.7 ± 332.7, respectively, p=0.84, n=4).

\textbf{Figure 1.} Box plots of the distribution of glutathione in controls and patients with CF. Each box separates the data into quartiles, with the central notches encompassing the middle 50 percent of the distribution (ie, the 25th and 75th percentiles). The horizontal line within the box represents the median while the vertical lines above and below ("whiskers") denote the extent of the middle 90 percent of the distribution. Samples beyond the whiskers represent the outlying 5 percent of the data values. Individual data are indicated by dots. The mean ± SD for each group is correspondingly printed below the horizontal axis. There was a significantly higher variability in the CF group values compared with the controls (p=0.01).

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\caption{Box plots of the distribution of glutathione in controls and patients with CF. Each box separates the data into quartiles, with the central notches encompassing the middle 50 percent of the distribution (ie, the 25th and 75th percentiles). The horizontal line within the box represents the median while the vertical lines above and below ("whiskers") denote the extent of the middle 90 percent of the distribution. Samples beyond the whiskers represent the outlying 5 percent of the data values. Individual data are indicated by dots. The mean ± SD for each group is correspondingly printed below the horizontal axis. There was a significantly higher variability in the CF group values compared with the controls (p=0.01).}
\end{figure}
inflammation of CF. Overcome by the large number of neutrophils present in the epithelial lining fluid (which may be as high as 100- to 1,000-fold that of normal subjects), the bronchial oxidant-antioxidant balance becomes disrupted. As a result, antioxidants like GSH become depleted, and antiproteases like α1-antitrypsin become unprotected against oxidative inactivation. The oxidation of α1-antitrypsin by polymorphonucleated cells has been demonstrated in the sputum of patients with CF, leading to unopposed elastolytic destruction of the airways. Indeed, many of the pathologic changes observed in patients with CF are consistent with chronic uninhibited proteolysis and elastolysis.

The concentration of erythrocytic antioxidants, particularly the GSH system, has been extensively studied in patients with CF and found to be either increased or unchanged. To the best of our knowledge, however, no other studies have so far attempted a correlation between the concentration of RBC GSH and the extent of functional severity in patients with CF. The correlation demonstrated by our data could thus provide an explanation for the large interindividual variability of the GSH system activity. Patients with worse function (and possibly a greater oxidant burden) would be more likely to exhibit higher levels of RBC GSH than patients with mild dysfunction.

Our rationale for measuring only antioxidant con-

**FIGURE 2.** Correlation between erythrocytic GSH concentrations and percent predicted values of FVC in 32 patients with CF. A significant negative correlation was found (r = -0.35, p = 0.035).

**DISCUSSION**

These data confirm the results of previous studies suggesting that RBC antioxidants may act as somatic scavengers in situations of oxidative stress. We speculated that this novel RBC function might correlate with the degree of pulmonary deterioration in patients with CF. Given the erythrocytes' ubiquitous nature, their high antioxidant capacity, and the anatomic characteristics of the pulmonary microcirculation, RBC antioxidants appeared potentially well suited to represent a functional marker for the extent and severity of oxidative exposure in a chronic inflammatory process such as CF. Moreover, RBC antioxidants have been demonstrated capable of preventing the oxidative inactivation of α1-antitrypsin by freshly prepared cigarette smoke and phorbol-stimulated phagocytes. Thus, there was enough ground to speculate that they might have also played a protective role in CF, a disease characterized by the presence of large amounts of oxidized and inactive α1-antitrypsin in the epithelial lining fluid. Our results lend support to this premise and suggest that GSH concentrations may provide a clinical marker for the functional severity of patients with CF.

Oxidants released on the surface of the bronchial epithelium by neutrophils and macrophages, represent a major component of the chronic airway

**FIGURE 3.** Correlation between erythrocytic GSH concentrations and percent predicted values of FEV1 in 32 patients with CF. A significant negative correlation was found (r = -0.30, p = 0.047).
centnation and not the "oxidant burden" was based on the day-by-day variability of oxidative stress caused by recurrent infections and exacerbations. Indeed, the correlation reported by Meyer and Zimmerman\(^\text{20}\) between the intra-airway release of neutrophil proteases/pro-oxidants and the degree of pulmonary dysfunction of patients with CF was measured during periods of respiratory exacerbations. Thus, one baseline observation might not have accurately reflected the "cumulative" oxidative stress suffered by the airways of our patients. Moreover, because the measurement of intra-airway oxidants is technically cumbersome and requires a bronchoscopy, the assay would not have lent itself to a possible routine use. We hypothesized that antioxidant concentrations of peripheral erythrocytes might have provided a more accessible, albeit indirect, indicator for the "cumulative" damage suffered by patients with CF. Our data support this premise.

In summary, patients with CF having more severe respiratory dysfunction appear to have a compensatory increase in their intracellular RBC GSH concentration when compared to patients with CF having mild respiratory dysfunction and age-matched controls. Thus, there appears to be a compensatory, but probably inadequate, increase in erythrocytic antioxidants. Further evaluation of the role of RBC GSH as a "biologic marker" of CF severity could be carried out by comparing baseline concentrations with levels measured during intercurrent respiratory infections, antibiotic treatment, or aerosolized administration of GSH.

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