Systemic Antioxidants and Lung Function in Asthmatics during High Ozone Season: A Closer Look at Albumin, Glutathione, and Associations with Lung Function

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Abstract

Background: Asthma is a chronic airway inflammatory disease with episodic symptoms of wheezing, chest tightness, cough, and shortness of breath. High ambient ozone levels have been associated with increased airway inflammation and asthma morbidity in prior studies. Mechanisms underlying individual susceptibility to asthma exacerbations from poor air quality are not fully understood.

Objective: As part of a panel observational study, we hypothesized that systemic antioxidant ability and antioxidant status may be associated with more stable asthma during high ozone season.

Methods: A cross sectional study was performed to evaluate the antioxidant profile in systemic circulation and its associations with clinical parameters in asthmatics and healthy controls during three summers in Atlanta, Georgia.

Results: In this panel of individuals with and without asthma, we found that although systemic glutathione levels were not different between the groups, serum albumin was significantly lower in the asthmatic group. Albumin also significantly correlated with lung function (%FEV₁) and asthma quality of life scores. In a subgroup tested, plasma reduced glutathione (GSH) levels were associated with worse airways obstruction.

Conclusion: Antioxidants GSH and albumin may have a role in maintaining lung function and asthma stability during times of poor ambient air quality. Clin Trans Sci 2014; Volume 7: 314–318

Keywords: ozone, asthma, antioxidants, albumin, glutathione, lung function

Introduction

Asthma is a chronic airway disease which manifests clinically as episodic symptoms of wheeze, chest tightness, cough, and shortness of breath.^{1,2} Exposure to various triggers may initiate this inflammatory and oxidant cascade. One such exposure is poor outdoor air quality.^{3–5} The United States Environmental Protection Agency (USEPA) has determined standards for certain criteria pollutants such as atmospheric ozone to protect public health. However, Atlanta, Georgia has historically realized ozone levels above those standards from May through September due to the combination of heat, sunlight, and high emissions of volatile organic compounds (VOCs).⁶ The association of poor air quality and worsening asthma was underscored by a study during the 1996 Summer Olympics in Atlanta. Reduced traffic congestion due to changes in traffic patterns and use of public transportation significantly diminished atmospheric ozone levels in the city. The temporal reduction in air pollution was associated with fewer acute asthma visits among children.⁷

Not all individuals appear to be equally affected by poor air quality. Reduced antioxidant levels and/or activity have been associated with certain lung conditions such as asthma, and prior studies have suggested that antioxidant profiles may be protective during times of poor air quality.^{8,9} For this observational cross-sectional study of asthmatics and nonasthmatics living in the Atlanta area, we considered poor air quality from high ozone season as the common exposure. We hypothesized that systemic and upper airway antioxidant ability may be associated with maintaining lung function in a group of asthmatics during high ozone season.

Methods

As described previously, healthy controls and clinically stable asthmatics were enrolled in a cross-sectional observational study during high ozone season.¹⁰ After reviewing and providing

informed consent, participants residing in the metropolitan Atlanta area were enrolled during high ozone season (May–September) in 2003, 2005, and 2006. Asthmatics included were at least 18 years old, nonsmokers (less than 5 pack-year smoking history more than 5 years ago), lived in metropolitan Atlanta, and did not take vitamin supplements. Individuals who were acutely ill or had received systemic corticosteroids within the prior 2 weeks were excluded from participation. The definition and severity of asthma was based on clinical history and presence of airflow obstruction and/or documentation of a significant bronchodilator response.² Healthy controls were nonsmokers with no history of pulmonary disease or allergic rhinitis and normal spirometry. The study was supported by a K12 grant from NIH National Center for Research Resources K12 RR 017643, and Emory University Research Committee grant, and a private grant from The Carlyle Fraser Heart Center, Emory Crawford Long Hospital.

Clinical visits

Clinical parameters collected included a medical history, the Juniper asthma quality of life questionnaire (QOLQ), and a peripheral blood sample for leukocyte, eosinophil, and IgE levels.¹¹ A change in asthma QOLQ scores >1.0 are validated as a moderately meaningful difference and >1.5 are considered a large difference in perceived quality of life.¹² Spirometry and offline exhaled nitric oxide level measurements (eNO) were performed according to American Thoracic Society standards.^{13,14} Exhaled NO samples were analyzed by chemiluminescence analyzer (Sievers 280i, Boulder, CO, USA). For this study, plasma antioxidants albumin and uric acid were measured in the clinical laboratory. Nasal lavage was performed by spraying 4 cc of normal saline into each nostril using a handheld nebulizer that delivered 100 mL per actuation.¹⁵ Each lavage consisted of eight sets of

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five sprays. The lavage was recovered by forceful expulsion into a specimen cup immediately after each set of five actuations. The specimen was centrifuged, filtered, and divided into aliquots and stored in a study freezer at -70°C until analysis.

High-performance liquid chromatography (HPLC) measurement of thiols

Nasal lavage and plasma samples were preserved immediately after collection in a 5% perchloric acid solution containing iodoacetic acid ($6.7\ \mu\text{M}$) and boric acid ($0.1\ \text{M}$). The preservation fluid also contained $5\ \mu\text{M}$ of the internal standard γ -glutamyl-glutamate (γ -Glu-Glu). This step was completed within 30 minutes of collection to prevent degradation or oxidation of GSH. After protein removal, samples were derivatized with dansyl chloride and separated on a $10\text{-}\mu\text{m}$ Ultrasil amino column by HPLC (Waters 2690; Waters Corp., Milford, MA, USA). Fluorescence detection was recorded by two detectors (Waters 474 and Gilson model 121, Gilson, Inc., Middleton, WI, USA). The concentrations of GSH and GSSG were calculated by quantitating the integrated areas relative to that of γ -Glu-Glu. Dilution due to the lavage procedure was corrected using the urea method.¹⁶ The concentration of urea in all the samples was obtained with an adopted Berthelot method from Pointe Scientific, Inc. (Canton, MI, USA) and has a sensitivity range of $0.05\text{--}150\ \text{mg/dL}$. The urea dilution factor is equal to $[\text{urea}]_{\text{plasma}}/[\text{urea}]_{\text{lavage fluid}}$.¹⁷

Statistical analysis

Nonparametric tests (Wilcoxon rank-sum) were used to evaluate differences between healthy controls and asthmatics with respect to quantitative measures such as lung function, laboratory values, and antioxidant levels. Linear regression modeling with biologically plausible and consistent models, excluding collinear variables, was used to measure associations of clinical parameters with antioxidant levels. Model variables were selected due to the plausible and likely independent contributions of each variable to the outcome measure. Statistical analyses were performed with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA), with p values ≤ 0.05 considered significant.

Results

During high ozone seasons from 2003, 2004, and 2006, 38 asthmatics of varying severity (18 mild, 13 moderate, and 6 severe by Global Initiative for Asthma criteria for airway obstruction) and 13 nonasthmatic controls were enrolled. Number of days each year with 8-hour averaged ozone exceedances (greater than $0.080\ \text{ppm}$) were 13, 11, and 30, respectively.¹⁸ Median 8-hour averaged ozone levels for the 2 days prior to presentation of these participants was $0.061\ \text{ppm}$ and have been described previously.¹⁰ Of these individuals, 31 provided plasma samples and 14 asthmatics (four severe, two moderate, and eight mild asthmatics) had reliable upper airway lavage samples available for antioxidant measurements. As reported previously and to provide context, characteristics of participants are presented in Table 1 and demonstrate expected differences in lung function, markers of inflammation such as leukocyte count and neutrophilia, IgE levels, and exhaled NO levels.

Antioxidants, lung function, and clinical parameters

Antioxidant glutathione (GSH), oxidized glutathione (GSSG) levels, and percent oxidized glutathione ($\text{GSSG}/[\text{GSSG} + \text{GSH}]$) in the systemic circulation were not significantly different between the asthmatic and nonasthmatic group. However, plasma levels of

albumin were significantly lower in the asthmatic as compared with the nonasthmatic group. Among asthmatic patients during high ozone season, higher plasma GSH levels was associated with lower FEV_1 %predicted ($R = -0.404$, $p = 0.045$; Figure 1) and lower %predicted FEV_1/FVC ratio ($R = -0.461$, $p = 0.020$; Figure 2).

Plasma albumin levels directly correlated with % FEV_1 among asthmatics ($R = 0.378$, $p = 0.010$; Figure 3) though not with % FEV_1/FVC ratio. Albumin levels also correlated with %oxidized GSH (GSSG) in asthmatics $R = 0.55$, $p < 0.01$ (Figure 4) though less so in all participants in the study ($R = 0.44$, $p = 0.02$). In asthmatics, this relation was preserved even after controlling for % FEV_1 (Table 2a). Interestingly, the asthma QOLQ score was more strongly associated with systemic albumin concentrations than measures of airflow: Controlling for % FEV_1 , an increase of $1\ \text{g/dL}$ in albumin was associated with a clinically significant 1.8 higher QOL score (95% C.I. 0.71 , 2.90 ; Table 2b). There were no other significant associations with clinical factors such as peripheral eosinophilia or IgE levels.

Relationships of systemic and airway antioxidants

In the group of asthmatics with upper airway and plasma sampling, there was not a direct but an inverse correlation of GSH levels between the compartments: upper airway GSH levels inversely correlated to systemic GSH levels (asthma $R = -0.72$, $p = 0.004$). Interestingly, systemic levels of oxidized fraction of glutathione (%GSSG) correlated with upper airway levels of GSH (all $R = -0.744$, $p = 0.0009$).

Discussion

This study demonstrates that, particularly in asthmatics, lung function has an inverse association with plasma reduced GSH and a direct correlation with albumin levels, underscoring the hypothesis that antioxidant availability and capability is important in asthma.^{19,20} However, airway and systemic levels of GSH are inversely associated, suggesting that antioxidant adaptations in the body are compartmentalized. Our study also suggests that the nonenzymatic antioxidant anti-inflammatory properties of systemic albumin play a role in maintaining lung function.

The pathophysiology of asthma is one of increased inflammation-related oxidative stress, particularly during exacerbations.¹⁹ During these episodes, asthmatics have demonstrated pro and antioxidant alterations, which can make interpreting antioxidant levels difficult. The lung has multiple layers of defense against persistent oxidant stress.²¹ Glutathione and albumin, antioxidants of interest in this study, are both nonenzymatic antioxidants. GSH is a tripeptide which comprises of a thiol group. The oxidation of glutathione is necessary to reduce the reactive oxidant species hydrogen peroxide (H_2O_2) to H_2O via glutathione peroxidase (GPx). In turn, glutathione reductase (Gr) reduced the oxidized/disulfide form of glutathione (GSSG) to replenish antioxidant capacity. The majority of the total pool of glutathione is in oxidized form (GSSG) despite no difference in GPx activity between asthmatics and healthy controls, as severe asthmatics exhibit lower total GSH in epithelial lining fluid (ELF) collected via BAL.^{20,21} Higher GSSG levels even in sputum are markers of oxidant stress in lung disease. In a cohort of severe pediatric asthma patients, percent oxidized glutathione (GSSG/GSH + GSSG) predicted exhaled NO levels and hospitalizations over the prior year.²⁰ In our study, asthmatics GSH antioxidant ability in systemic circulation is higher in association with worse airways obstruction, possibly indicating a protective or adaptive response to oxidative stress.

Asthmatics			Controls		p value*
(a)					
N	38	(31–50)	13	(29–55)	0.65
Age	44		40		
Gender F: M	33 : 5		11 : 2		
Race C/AA/As/O	11/26/0/1		4/8/1/0		
BMI	32	(24.5–40.4)	29.8	(22.7–40.6)	0.28
Lung function					
%FEV ₁ /FVC ratio	91	(81–101)	97	(96–102)	0.04
%FEV ₁	79	(69–90)	88	(82–94)	0.06
%PEFR	78	(66–99)	86	(66–88)	0.51
Exhaled NO (ppb)	11.4	(9.8–17.0)	7.1	(6.3–12.5)	0.02
Blood leukocytes (× 10 ³)	6.8	(5.7–8.6)	5.3	(4.8–6.4)	0.07
Eosinophils (%)	2	(2–4)	2	(1–2)	0.06
Neutrophils (%)	62	(53–68)	53	(41–62)	0.08
IgE (IU/mL)	134	(50–324)	34	(14–74)	<0.01
(b)					
38 Asthmatics and 13 controls					
Albumin (g/dL)	3.7	(3.5–4.0)	4.0	(3.8–4.4)	0.03
Uric acid (mg/dL)	4.3	(4.0–5.9)	4.2	(3.8–5.2)	0.45
Plasma values are in subset: 25 asthmatics and 6 controls					
GSH (uM)	0.350	(0.196–0.749)	0.405	(0.282–0.557)	0.92
GSSG (uM)	0.608	(0.062–1.234)	0.567	(0.041–1.516)	0.45
%GSSG	0.426	(0.169–0.652)	0.391	(0.126–0.720)	0.45
NL GSH (uM)	0.216	(0.158–0.398)			
NL GSSG (uM)	1.548	(0.856–2.598)			
NL %GSSG	0.850	(0.804–0.899)			
Data presented and median and IQR; *p value from Wilcoxon Rank Sum Test. Source for Table 1(a): Khatri et al. ¹⁰					

Data presented and median and IQR; *p value from Wilcoxon Rank Sum Test. Source for Table 1(a): Khatri et al.¹⁰

Table 1. (a) Demographics of study participants. (b) Systemic and airway antioxidant levels. C = Caucasian; AA = African American; As = Asian; O = other; FEV₁ = forced expiratory volume in 1 second; FVC = forced vital capacity; PEFR = peak expiratory flow rate; NO = nitric oxide; GSH = reduced glutathione; GSSG = oxidized glutathione; NL = nasal lavage.

Figure 1. Among asthmatics during high ozone season, plasma GSH levels inversely correlated with %Ratio. ($R = -0.40$, $p = 0.05$)

Figure 2. Among asthmatics during high ozone season, plasma GSH levels inversely correlated with %FEV₁. ($R = -0.46$, $p = 0.02$)

Figure 3. Plasma albumin levels correlate with lung function in asthmatics during high ozone season. ($R = 0.378$, $p = 0.01$)

Figure 4. Among asthmatics during high ozone season, percent oxidized glutathione correlated with albumin. ($R = 0.55$, $p < 0.01$)

	Parameter estimate	95% CI	p value
(a)			
Intercept	2.904	2.174, 3.636	<0.0001
%GSSG	0.830	0.315, 1.345	0.002
%FEV ₁	0.006	−0.003, 0.016	0.1881
(b)			
Intercept	−2.459	−6.489, 1.571	0.242
Albumin (g/dL)	1.803	0.707, 2.899	0.004
%FEV ₁	0.008	−0.018, 0.034	0.539

Table 2. (a) Plasma albumin levels correlated with %GSSG in asthma, controlling for %FEV₁ ($p < 0.01$). (b) Asthma QOL score was associated with albumin more than %FEV₁ ($p = 0.004$). FEV₁ = forced expiratory volume in 1 second; GSSG = oxidized glutathione.

Albumin has been noted to be a substantial (approximately 28%) contributor to systemic antioxidant capability as well as having anti-inflammatory functions.²² Availability, coordination, and synthesis of GSH and albumin appear to be related. Albumin is the most abundant multifunctional plasma protein in the intravascular space, constituting approximately 60% of total plasma protein. Antioxidant ability of albumin arises from ligand-binding capacities, as with its ability to bind to transition metals such as copper and iron as well as long-chain fatty acids. Albumin also works in conjunction with bilirubin and vitamin E to inhibit oxidation of low density lipoprotein cholesterol. Due to the high amount of albumin in plasma, the reduced cysteine residue (Cys 34) on molecular albumin collectively constitutes the largest pool of thiols in circulation.²³ Particularly relevant to findings in this study, albumin is believed to be an important antioxidant regulating glutathione activity in lung epithelial cells, and deficiency of albumin likely diminishes individuals responses to oxidant stress. Albumin may also modulate GSH synthesis (through gamma-glutamylcysteine synthetase) gene expression. Similar to other prior work, our study also demonstrated that asthmatics had significantly lower systemic albumin levels than their healthy counterparts.²² In addition, we demonstrate a significant correlation between albumin and %FEV₁, which is

similar to another study of dietary antioxidant intake and systemic levels, in which a significant positive correlation has been noted between serum albumin and lung function ($R = 0.36$, $p < 0.0001$).²⁴ Therefore, in contrast to systemic oxidized GSH, which provided a picture of ongoing oxidative stress, albumin was associated with preserved lung function.

Finally, upper airway GSH levels inversely correlated with systemic GSH levels, demonstrating the lack of a parallel association in airway as compared to systemic antioxidant profile. Conceivably, all individuals upregulate airway antioxidants in response to persistent oxidant stress (ozone). Systemic levels of oxidized fraction of glutathione (%GSSG) correlated with upper airway levels of GSH which suggests that upper airway levels of total antioxidants or antioxidant ability was associated with higher proportion of oxidized antioxidants systemically. These findings underscore the independent dynamics of the airway and systemic antioxidant defenses or that higher levels of reduced antioxidants may be available at the location of highest oxidant stress.²⁵

Limitations to this observational study approach to evaluate antioxidant status and air pollution in asthmatics are inherent. High ozone season in Atlanta was assumed to be a unifying air pollution exposure. Actual measurement of individual exposures can be technically challenging and was outside the scope of

this study. Our previous work in asthmatics during this period demonstrated that ambient ozone levels, though not always exceeding the set air quality standards, did have associations with allergies, symptoms, and eosinophilia.¹⁰

Conclusion

This real-world observational study evaluating airway and systemic antioxidant status and lung function in asthmatics during high ozone season demonstrated that the innate GSH antioxidant profile in asthmatics support asthma stability and lung function. Due to the potential biochemical interaction between GSH and albumin and the noted association with lung function, systemic albumin appears to be associated with the glutathione antioxidant pathway. Finally, respiratory and systemic antioxidant levels demonstrated different profiles during times of increased oxidant stress. This panel study underscores the importance of antioxidant ability in asthmatics to maintain asthma stability.

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