

Systemic oxidative and antioxidative status in Chinese patients with asthma

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Background: Patients with asthma generate an increased amount of reactive oxygen species from peripheral blood cells. Reactive oxygen species produce many of the pathophysiologic changes associated with asthma and may contribute to its pathogenesis.

Objective: We investigated changes in antioxidant enzyme activities and oxidized glutathione (glutathione disulfide; GSSG) levels in erythrocytes from a group of healthy control Chinese subjects ($n = 135$) and patients with asthma ($n = 106$).

Methods: Baseline pulmonary function was measured for all subjects. Antioxidant status was evaluated by measuring erythrocyte superoxide dismutase, catalase, and glutathione peroxidase activities. Oxidative stress was also measured in terms of GSSG in erythrocytes with a kinetic microassay.

Results: Patients with asthma had significantly increased erythrocyte superoxide dismutase and catalase activities compared with controls (61.10 ± 1.30 U/g hemoglobin [Hb] vs 55.51 ± 1.82 U/g Hb [$P = .018$] and 0.0637 ± 0.0021 U/g Hb vs 0.0257 ± 0.0120 U/g Hb [$P < .001$] for the asthma and control groups, respectively). Conversely, erythrocyte glutathione peroxidase activity decreased (44.21 ± 1.33 mU/g Hb vs 50.07 ± 1.39 mU/g Hb for the asthma and control groups, respectively; $P = .003$). Patients with asthma also had significantly higher GSSG levels in erythrocyte hemolysates compared with controls (167.40 ± 2.93 $\mu\text{mol/L}$ vs 44.98 ± 0.44 $\mu\text{mol/L}$ for the asthma and control groups, respectively; $P < .001$), indicating increased oxidative stress.

Conclusions: Asthma is accompanied by an alteration in systemic antioxidant status due to possible oxidative stress in this disease. (*J Allergy Clin Immunol* 2004;114:260-4.)

Key words: Asthma, superoxide dismutase, catalase, glutathione peroxidase, erythrocyte, oxidized glutathione, oxidative stress

Asthma is a chronic inflammatory airway disease that affects children and adults of all ages.^{1,2} Although the pathogenesis of asthma remains incompletely defined, it has been shown to be a state of increased free radical

Abbreviations used

BAL: Bronchoalveolar lavage
GPx: Glutathione peroxidase
GSH: Reduced glutathione
GSSG: Glutathione disulfide, or oxidized GSH
Hb: Hemoglobin
H₂O₂: Hydrogen peroxide
NADPH: Nicotinamide adenine dinucleotide phosphate
ROS: Reactive oxygen species
SOD: Superoxide dismutase

formation. Cells obtained from the peripheral blood and lungs of patients with asthma generate increased amounts of reactive oxygen species (ROS), such as superoxide radicals and hydrogen peroxide (H₂O₂), and the increase correlates with disease severity.³⁻⁵

The lungs and blood possess a wide array of enzymatic and nonenzymatic antioxidant defenses, including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and reduced glutathione (GSH). In several studies, whole blood, plasma, platelets, peripheral blood cells (neutrophils), and bronchoalveolar lavage (BAL) cells obtained from patients with asthma have exhibited reduced antioxidant defenses, including reduced GPx and SOD activities.⁶⁻¹³ Conversely, GSH, the most abundant non-protein thiol in cells, plays a prominent role in antioxidant protection of the lung. In humans, the GSH concentrations in the epithelial lining fluid are normally much higher than in blood.¹⁴ In the GSH redox cycle, the measurement of oxidized glutathione (glutathione disulfide; GSSG) in tissues and biological fluids has been used as an index of the oxidative stress that occurs under different physiological and pathologic conditions.^{15,16} GSSG has been reported to be higher in the epithelial lining fluid of patients with adult respiratory distress syndrome¹⁷ and in the bronchial wash and BAL of asthma patients.¹⁸ There was also a tendency toward higher levels of GSSG in induced sputum of patients with asthma.¹⁹ Determination of the systemic GSSG may be useful during investigation of oxidative stress in the lung.

In the studies performed so far, changes in only a few variables or a small number of subjects have been evaluated. To define the systemic antioxidant defenses in asthma, GSSG concentrations and SOD, catalase, and GPx activities were measured in erythrocyte hemolysates from healthy subjects and from patients with asthma.

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Supported by grant HKU7223/01M from the Hong Kong Research Grant Council.

Received for publication March 26, 2004; revised April 28, 2004; accepted for publication May 5, 2004.

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0091-6749/\$30.00

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doi:10.1016/j.jaci.2004.05.013

TABLE I. Classification of asthma severity score

Score A	FEV ₁ % predicted		Score B	Medication usage		Combined score (A + B)	Asthma severity
0	>80	+	1	Bronchodilator only	=	1-2	Mild
1	70-80		2	< 800 µg of inhaled steroid		3-4	Moderate
2	60-69		3	>800-1600 µg of inhaled steroid		5-8	Moderate to severe
3	50-59		4	>1600 µg of inhaled steroid		—	—
4	< 50		—	—		—	—

TABLE II. Clinical and demographic characteristics of the study groups

Variable	Controls (nonsmokers)	Asthmatics	
		Nonsmokers	Smokers
n	135	106	25
Male/female	54:81	40:66	20:5
Age (y)	47.3 ± 1.3	42.0 ± 1.5*	39.0 ± 3.1
Skin prick test (positive/negative)	28:94	57:41	11:9
FEV ₁ (% predicted)	111.6 ± 1.3	83.5 ± 2.4†	76.8 ± 5.3
SOD	55.51 ± 1.82	61.10 ± 1.30‡	67.03 ± 2.77§
Catalase	0.0257 ± 0.0012	0.0637 ± 0.0021†	0.0617 ± 0.0038
GPx	50.07 ± 1.39	44.21 ± 1.33*	48.55 ± 2.84
GSSG (µmol/L)	44.98 ± 0.44	167.40 ± 2.93†	174.60 ± 5.89

**P* < .01.

†*P* < .001.

‡*P* < .05 compared with healthy controls.

§*P* < .05 compared with nonsmoking patients with asthma.

METHODS

Subjects

A total of 139 consecutive Chinese patients with asthma were recruited from the asthma clinic at Queen Mary Hospital in Hong Kong, of whom 106 patients were nonsmokers. They were all stable patients receiving treatment with inhaled corticosteroids, except for 2 patients who were receiving treatment with a bronchodilator only. Lung function tests were performed, and blood samples were taken. The patients were classified according to a combination of lung function (FEV₁ % predicted) score plus medication usage score into 3 categories of asthma severity (Table I), modified from a previous publication.²⁰

A total of 135 healthy nonsmoking subjects without respiratory symptoms and normal lung function served as controls. These individuals were recruited from a random population study to determine reference values of lung function tests in Hong Kong. Blood samples were also taken for determination of antioxidant status in this study. This study was approved by the Ethics Committee of The University of Hong Kong.

Blood collection and storage

Whole blood 10 mL in lithium heparin was taken from each subject and immediately centrifuged at 1600g. Erythrocytes and plasma were separated and stored at -70°C until biochemical measurements were taken. Hemoglobin (Hb) concentrations were assayed by a commercially available Hb kit (Sigma, St Louis, Mo).

Measurements of specific antioxidant enzyme activities and GSSG catalase activity. The quantitation of catalase activity in erythrocyte hemolysates was based on the reaction with H₂O₂, as previously described.²¹ In brief, the initial rate of disappearance of H₂O₂ (0-60 s) was recorded spectrophotometrically at a wavelength

of 240 nm. One unit of catalase activity was defined as the rate constant of the first-order reaction. The catalase activity is expressed as units per gram of Hb.

SOD activity. SOD activity in erythrocyte hemolysates was determined from the rate of reduction of cytochrome *c*,²² with 1 U of SOD activity defined as the amount of SOD required to inhibit the rate of cytochrome *c* reduction by 50%. Xanthine oxidase (Sigma) was added at a concentration sufficient to induce a 0.020 change in absorbance per minute at 550 nm. The SOD activity was expressed as units per gram of Hb.

GPx activity. Total GPx activity was determined spectrophotometrically in erythrocyte hemolysates through an indirect coupled assay.²³ The reactions were performed with the Bioxytech GPx-340 assay kit (Oxis, Portland, Ore). One unit of activity was defined as the activity that catalyzes the oxidation of 1 nmol of nicotinamide adenine dinucleotide phosphate (NADPH) per minute. A molar coefficient of extinction of 6.22 × 10⁶ L/mol/cm was used for NADPH. The GPx activity was expressed as milliunits per gram of Hb.

Measurement of GSSG. GSSG was measured by a new modified method as a kinetic microassay in erythrocyte hemolysates.²⁴ GSSG is reduced to GSH by the action of a highly specific glutathione reductase and NADPH in the presence of 5,5'-dithio-bis-(2-nitrobenzoic acid). A range of GSSG concentrations (2-10 µmol/L) were prepared as standards. The reaction mixture was equilibrated, and the kinetic reaction was observed for 5 minutes at 420 nm on a microplate reader. The samples were mixed for approximately 5 seconds, and the absorbance was read for 5 minutes. Samples were calculated from the standard curve to determine the GSSG concentration.

Analysis of data

Data are expressed as means ± SEMs. The unpaired *t* test was used to compare the control subjects and patients with asthma. The study

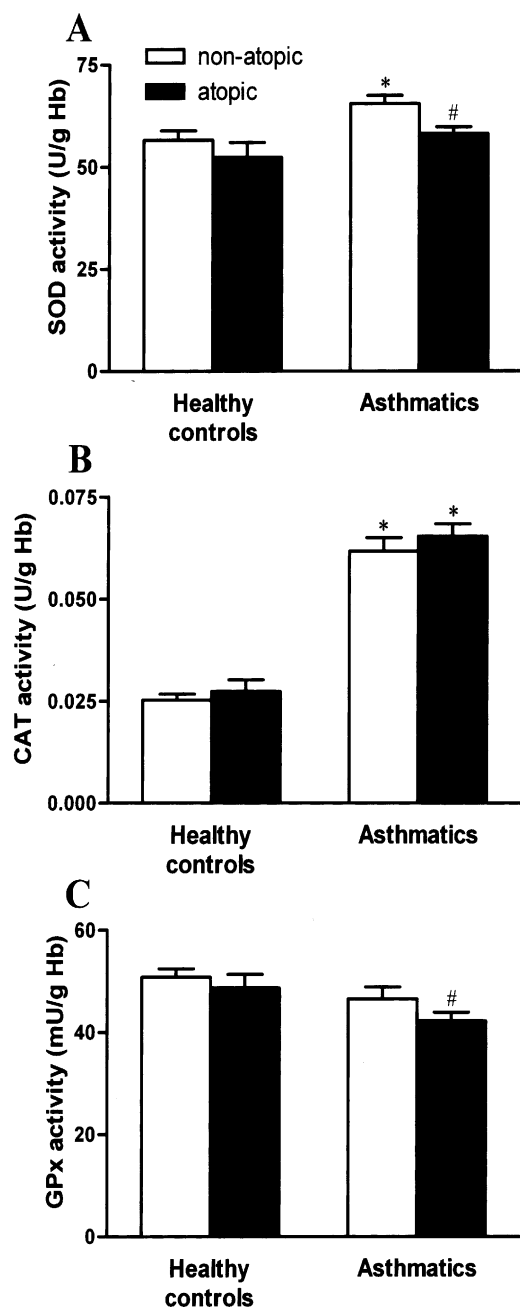


FIG 1. Variation of superoxide dismutase (SOD) (A), catalase (CAT) (B), and glutathione peroxidase (GPx) (C) activities in erythrocytes in nonatopic and atopic controls ($n = 122$) and patients with asthma ($n = 98$). All were nonsmokers. Means $\pm$ SEMs are shown. * $P \leq .01$ for comparison among nonatopic patients with asthma and nonatopic healthy controls, atopic patients with asthma, and atopic healthy controls; # $P \leq .05$ for comparison between atopic patients with asthma and nonatopic patients with asthma.

parameters were compared among patients with different asthma severity scores by ANOVA. The relationship between different study parameters and the degree of airway obstruction was evaluated by computing the Pearson correlation coefficient. The Spearman distribution rank correlation test was also used where appropriate.

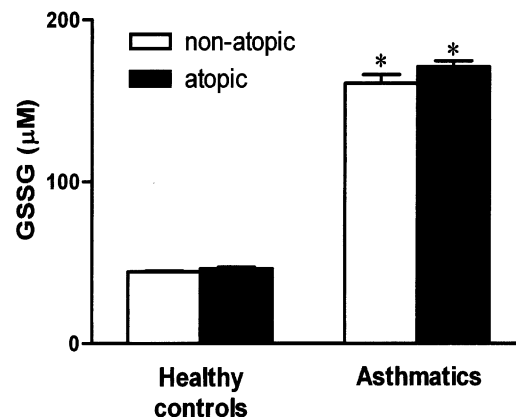


FIG 2. Variation of oxidized glutathione (glutathione disulfide; GSSG) levels in erythrocyte hemolysates in nonatopic and atopic healthy controls ($n = 122$) and patients with asthma ($n = 98$). All were nonsmokers. Means $\pm$ SEM are shown. * $P < .001$ for comparison among nonatopic patients with asthma and nonatopic healthy controls, atopic patients with asthma, and atopic healthy controls.

TABLE III. Correlation coefficients of lung function (FEV₁ % predicted), antioxidant enzyme activities, and GSSG levels in controls and patients with asthma

Study parameter	Correlation coefficients (<i>r</i>)	
	FEV ₁ of controls	FEV ₁ of asthmatics
SOD	−0.142	−0.083
CAT	−0.057	0.124
GPx	−0.067	0.008
GSSG	0.140	−0.137

Statistical analyses were performed with SPSS for Windows (Version 11.0; SPSS Inc, Chicago, Ill). A P value of $< .05$ was considered statistically significant.

RESULTS

Table II shows the clinical and demographic characteristics of the study subjects. Patients with asthma had a significantly lower FEV₁ (% predicted) than healthy controls. Regarding the antioxidant enzyme activities in erythrocyte hemolysates (Table II), the activities of SOD and catalase enzymes in patients with asthma were significantly enhanced compared with the levels in controls. Conversely, there were significant decreases in the mean value of GPx activity compared with the levels in controls. Significantly higher levels of GSSG were found in erythrocyte hemolysates from patients with asthma than in healthy controls (Table II); this indicates the presence of oxidative stress in the systemic circulation. Both erythrocyte catalase activity and the level of GSSG were significantly higher in patients with asthma than in healthy controls irrespective of atopic status (Fig 1, B and Fig 2). Erythrocyte SOD and GPx activities were significantly lower in atopic ($n = 57$) than nonatopic ($n = 41$) patients with asthma (Figure 1, A and C). In addition, significantly higher erythrocyte SOD

TABLE IV. Comparison of antioxidant activities and GSSG levels in erythrocyte hemolysates between patients with different severities of asthma

Study parameter	Mild (n = 26)	Moderate (n = 50)	Moderate to severe (n = 28)
SOD (U/g Hb)	66.65 ± 4.78	63.76 ± 2.86	69.17 ± 4.51
CAT (U/g Hb)	0.0651 ± 0.0056	0.0775 ± 0.0079	0.0658 ± 0.0052
GPx (mU/g Hb)	51.32 ± 4.09	45.98 ± 2.74	51.14 ± 4.47
GSSG (μmol/L)	158.03 ± 6.76	162.42 ± 5.30	172.29 ± 5.28

activity was found in nonatopic patients with asthma compared with nonatopic controls (Figure 1, A) and in smoking (n = 25) compared with nonsmoking patients with asthma (Table II). The mean FEV₁ (% predicted) was lower in smoking than in nonsmoking patients with asthma, but this did not reach statistical significance (Table II).

No significant correlation was observed between the different study parameters and the FEV₁ (% predicted) in either healthy controls or patients with asthma (Table III). We then compared the study parameters among patients with different asthma severity scores. No significant differences were observed for any of the parameters studied (Table IV). A trend of higher levels of GSSG was found among patients with severe asthma, but this did not reach a significant level ($P = .08$). In addition, correlation analysis showed that SOD activity correlated positively with GPx activity in controls ($r = 0.276$; $P < .01$) and patients with asthma ($r = 0.402$; $P < .001$). In patients with asthma, SOD activity also correlated negatively with GSSG level. This indicates that imbalance of the SOD/GPx ratio may contribute to the remarkable enhancement of GSSG levels in asthma. However, catalase activity showed a negative correlation with GSSG level in controls ($r = -0.364$; $P < .001$), but not in patients with asthma.

DISCUSSION

In this study, we found increased oxidative stress, as shown by increased GSSG concentrations from erythrocyte hemolysates of patients with asthma compared with healthy control subjects, in accordance with the observed increase of GSSG in bronchial wash and BAL fluid.¹⁸ This was accompanied by alterations in increased SOD and catalase activities and decreased GPx activity in erythrocyte hemolysates in patients with asthma, in agreement with previously reported findings in blood mononuclear cells from patients with allergy.²⁵ We chose to study the systemic antioxidant status in erythrocytes because blood is an easily available source and is an important pool of antioxidant defenses in the body.^{26,27}

We considered characterization of the systemic antioxidant defenses to be important. First, asthma is an inflammatory disease,^{1,2,28} the inflammatory cells obtained from the lungs of patients with asthma generate increased amounts of ROS,^{3-5,13,29,30} and ROS can reproduce the key abnormalities of asthma.³¹ Second, the results reported so far seem to be contradictory or inconclusive on systemic antioxidant defenses concerning the

activities of SOD, catalase, and GPx in patients with asthma.⁶⁻¹³

Several reports have found alterations in different antioxidants in asthma. The role of antioxidant defense mechanisms in asthma is still inconclusive. Decreased activity of GPx, an enzyme involved in the detoxification of H₂O₂, has been well documented.^{8,9,11,13} In our study, we also observed a significant decrease in erythrocyte GPx activity in patients with asthma compared with controls. This might arise as a result of deficiency of selenium, which is an important component in the antioxidative defense through GPx activity, but inactivation caused by ·OH and superoxide radicals might also play a role.^{8,9,32} GPx is essential for removing toxic lipid oxidation products and H₂O₂, which are continuously generated as a result of sequestration and infiltration of inflammatory leukocytes in the lung. Misso et al³³ have argued that reduced activity of this enzyme in blood may reflect mechanisms associated with the pathogenesis and severity of asthma. However, no differences were found in the mean values of GPx activity in our patients with different asthma severity. As a limitation of this study, we did not measure selenium levels in the blood of these patients.

Erythrocytes are thought to be active scavengers of ROS by virtue of their large antioxidant reservoirs.^{26,27} In this study, we found a significant increase in erythrocyte SOD and catalase activities in patients with asthma, whereas others could find a significant increase only in SOD activity, with no change in catalase activity between patients with asthma and controls.¹³ We also found significantly higher erythrocyte SOD activity in nonatopic patients with asthma compared with atopic patients with asthma, in contrast to a previous report.³⁴ However, others either could detect reduced SOD activity in lung cells or could not detect any significant change in erythrocyte SOD activity in asthma.^{11,12} The imbalance of the SOD/GPx ratio causes an increase of peroxide availability with the consequence of higher ROS production in blood. Therefore, the enhancement of SOD and catalase activities could act as a defense mechanism against radicals. Smoking patients with asthma with a lower FEV₁ (% predicted) have a significantly higher erythrocyte SOD activity than nonsmoking patients with asthma. Increased GSSG levels as a marker of oxidant stress show the importance of increased oxidation of GSH caused by the imbalance in antioxidant functions.

In this study, the measures of oxidative stress and antioxidant enzyme activities did not differ significantly

between patient groups according to the severity of asthma. Similarly, none of the measured indices had a significant correlation with the degree of airway limitation (FEV₁ % predicted) in patients with asthma. This may be due to our group of patients, who were all patients with stable asthma and who were not experiencing acute exacerbations. In a recent study, inhaled glucocorticoids were shown not to have any systemic action on antioxidant enzymes.³⁵

Our study is the first to examine the changes in several measures of antioxidant enzymes and oxidative stress in a large group of Chinese subjects in Hong Kong. Unlike previous studies that focused on only a small number of study subjects and a few parameters, we investigated alterations in a wide variety of antioxidant enzyme activities and measured the index of oxidative stress in erythrocytes. It is clear that there are abnormalities in erythrocyte antioxidant enzymes and oxidant stress in nonatopic and atopic patients with asthma. Increased oxidative stress is likely to contribute to perpetuation and amplification of the inflammatory response. It is uncertain whether exposure to oxidative stress in the airways can induce airway inflammation. However, exposure to ozone and *in vivo* generation of oxygen radicals have been shown to induce airway hyperreactivity and inflammation.^{36,37} In conclusion, the oxidant burden of inflammation, as in asthma, is sufficient to overwhelm the antioxidant defense systems by high levels of GSSG in patients with asthma. These findings may provide a basis for understanding the enzymatic mechanisms and the interrelationships among all these enzymatic activities in asthma.

We thank Kathy Chow for her assistance in data analysis.

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