

The Oxidant-Antioxidant Balance in Mild Asthmatic Patients

I. Hanta,¹ S. Kuleci,¹ N. Canacankatan,² and A. Kocabas¹

¹University of Cukurova, School of Medicine, Department of Chest Diseases, Balcali/Adana, Turkey

²University of Cukurova, School of Medicine, Department of Biochemistry, Balcali/Adana, Turkey

Abstract. We investigated the oxidant-antioxidant balance and the effect of inhaled corticosteroids on this balance in mild stable asthmatics. Included in the study were 30 mild asthmatic patients (11 male, 19 female, mean age (year) \pm SD: 35.1 ± 9.7) and 26 healthy adults (7 male, 19 female, mean age (year) \pm SD: 40.8 ± 13.3). In all study groups, the peripheral venous blood samples were taken for plasma malonyldialdehyde (MDA), a parameter of lipid peroxidation caused by the oxidants, and erythrocyte superoxide dismutase (SOD), an antioxidant enzyme. The mean plasma MDA level was lower in the asthmatic group (5.7 ± 1.2 nmol/ml) than in the healthy group (6.3 ± 1.7 nmol/ml); and the mean erythrocyte SOD level was higher in asthmatic group (1086.4 ± 247.4 U/gHb) than in the healthy group (1028.0 ± 230.0 U/gHb). However, there were no significant differences in measurements of both plasma MDA levels and erythrocyte SOD enzyme activities between the groups (respectively, $p = 0.1$ and $p = 0.4$). When asthmatic patients were divided into subgroups as “inhaled steroid user” and “no inhaled steroid user”, no significant differences were observed in the measurements of either plasma MDA level or erythrocyte SOD enzyme activity between the mentioned subgroups. According to the results of our study, we can say that oxidant-antioxidant balance is not significantly affected in mild asthmatics or measurement of plasma level of MDA and erythrocyte SOD enzyme activity is not sensitive to the oxidant-antioxidant balance in mild asthmatics.

Key words: Asthma—Oxidative stress—MDA and SOD

Introduction

Asthma is a disease of unknown etiology, characterized by the chronic inflammation of airways. Although genetic and environmental factors may have a role in the development of asthma, the exact mechanisms of these factors are not clearly understood.

Oxidative stress, defined as exposure to excessive oxidants and/or reduced antioxidant capacity, is thought to contribute to the development of many diseases, especially chronic obstructive lung disease and asthma [18]. Eosinophils and other inflammatory cells found in the airways of asthmatic patients are the important sources of endogenous oxidants [3]. It is reported that oxidants might cause airway hyperreactivity, mucus hypersecretion, mucosal edema, and epithelial damage in asthmatics [16]. In patients with mild-to-moderate asthma and exacerbation of asthma, it has been revealed that plasma level of malonyldialdehyde (MDA), which is a product of lipid peroxidation caused by oxidants, increases in both bronchoalveolar lavage fluid and peripheral blood samples [15, 17]. Although a decrease in the enzymatic activity of erythrocyte superoxide dismutase (SOD) has been reported [5], there is another study in which erythrocyte SOD enzymatic activity has been increased [13]. It has been reported that markers such as hydrogen peroxide (H_2O_2), 8-isoprostane, nitric oxide (NO) and carbon monoxide (CO), reflecting oxidative stress, were higher in expired air of asthmatic patients [4, 12].

The inhaled corticosteroids (ICS) widely used in asthma treatment cause a decrease in products of lipid peroxidation by inhibiting airway inflammation [4, 15]. However, some studies have revealed that ICS might increase the enzymatic activity of SOD in airway epithelial cells and the production of glutathione in liver [16, 17]. Yet, reliable methods are limited *in vivo* evaluation of oxidant-antioxidant balance in asthma [3].

In this study, we aimed to evaluate the oxidative stress and the effect of ICS treatment on the oxidant-antioxidant balance in mild asthmatic patients.

Method

Thirty mild asthmatic adults were recruited from the outpatient clinic of the Department of Chest Diseases, Cukurova University, School of Medicine, and 26 healthy adults served as controls. Asthma was diagnosed by a chest physician who depended on convenient clinical and laboratory findings. Those patients having exacerbations of asthma and/or an additional disease and the patients who smoke were excluded. Of the 30 asthmatic patients, 23 had been using an ICS for the last month, whereas 7 patients had not during this period. In the first phase, clinical and demographical data of the study groups were obtained and physical examination, pulmonary function tests and skin prick tests were performed. Enduration larger than 3 mm in diameter to any of the allergens used in skin prick test was defined as atopy (Table 1). In the second phase, the peripheral blood sample was obtained in order to measure the erythrocyte SOD enzyme activity and plasma MDA level in both asthmatic and healthy groups.

Measurement of Erythrocytes SOD Enzyme Activity and Level of Plasma MDA

Whole blood was centrifuged at 3000 rpm for 10 min at 4°C. After plasma was separated for MDA determination, erythrocytes were washed with saline four times. SOD enzyme activity was assayed

Table 1. The clinical and demographic characteristics of the study groups

Characteristic	Patients with asthma (n:30)	Healthy controls (n:26)
Age	35.1 ± 9.7	40.8 ± 13.3
Female/Male	19/11	19/7
IgE (mg/dl)*§	509.4 ± 587.2	109.0 ± 123.8
Skin prick test (+)	27 (90%)	Not performed
FEV ₁ (L)*	2.5 ± 0.6	2.6 ± 0.5
FEV ₁ (predicted %)*	83.3 ± 17.6	90.5 ± 15.5
FEV ₁ /FVC (%)*§	73.0 ± 8.5	85.0 ± 5.6
FEV ₁ /FVC (predicted %)* §	86.1 ± 10.0	100.0 ± 6.6

FEV₁; forced expiratory volume in one second

FVC; forced vital capacity

*Mean ± standard deviation (±SD)

§*p* < 0.05

according to the methods of Mc Cord et al. in erythrocytes [10]. The lipid peroxide level in blood was assayed in terms of TBARS/MDA using 1,1,3,3-tetramethoxypropane as standard. The lipid peroxidation was expressed as nmoles of MDA formed per ml plasma [11]. The study was approved by the ethical committee of Cukurova University.

Statistical Analysis

SPSS for Windows 9.0 was used. Student *t* test and Mann Whitney U test were used for analysis of the differences between the groups.

Results

In this study, 30 adult mild asthmatic patients (11 male, 19 female, mean age ± SD: 35.1 ± 9.7) and 26 healthy adults (7 male, 19 female, mean age ± SD: 40.8 ± 13.3) were evaluated. Of the 30, 27 (90%) had a positive skin prick test. Mean FEV₁ (±SD) of asthmatic patients (83.3 ± 17.6, % predicted) was lower than the mean FEV₁ (±SD) of the healthy group (90.5 ± 15, % predicted) (*p* = 0.07) (Table 1).

Although the mean plasma MDA level was lower in asthmatic patients (±SD) (5.7 ± 1.2 nmol/ml) than in the healthy group (±SD) (6.3 ± 1.7) and mean erythrocyte SOD enzyme activity was higher in asthmatic patients (±SD) (1086.4 ± 247.4 U/gHb) than in the healthy group (±SD) (1028.0 ± 230.0 U/gHb), there was no significant difference in either comparisons (*p* > 0.05) (Table 2).

When asthmatic patients were divided into two subgroups according to inhaled steroid usage, there was no significant difference between 23 asthmatic patients using regular ICS and 7 asthmatic patients not using ICS, for mean plasma MDA level (±SD), respectively, 5.7 ± 1.2, 5.7 ± 1.3 nmol/ml, and mean erythrocyte SOD enzyme activity (±SD), respectively, 1068.0 ± 268.5, 1138.7 ± 180.6 U/gHb (*p* > 0.05) (Table 3).

Table 2. Plasma MDA levels and erythrocyte SOD enzyme activities of the study groups

Parameters	Patients with asthma (n:30)	Healthy controls (n:26)
SOD (U/gHb)* [¶]	1086.4 ± 247.4	1028.0 ± 230.0
MDA (mol/ml)* [¶]	5.7 ± 1.2	6.3 ± 1.7

*Mean ± standard deviation (SD)

[¶]*p* > 0.05**Table 3.** Plasma MDA levels and erythrocyte SOD enzyme activities of asthmatic patients who use ICS and those who do not

Parameters	Patients who use ICS (n:23)	Patients who do not use (n:7)
SOD (U/gHb)* [¶]	1068.0 ± 268.5	1138.7 ± 180.6
MDA (nmol/ml)* [¶]	5.7 ± 1.2	5.7 ± 1.3

*Mean ± standard deviation (SD)

[¶]*p* > 0.05

Discussion

Related literature has revealed that oxidative stress has an important role in the pathogenesis of asthma, and oxidants cause lipid peroxidation by oxidizing fatty acids and polyunsaturated lipids which are present in cell membranes. Thus, MDA, which is a marker of lipid peroxidation, increases [10]. Rahman et al. [17] has reported that plasma MDA level was higher in both asthmatic patients than in healthy controls, and in patients with asthma exacerbation than in patients with stable asthma. Similarly, in another study, MDA level in bronchoalveolar lavage fluid was higher in mild to moderate asthmatic patients than in healthy controls [15].

In mild asthmatic patients analyzed in this study, although the mean plasma MDA level was lower than in the healthy adults, there was no significant difference statistically. Because asthmatic patients in this study were stable and mild, it is assumed that their oxidative stress might not be intense. However, there is a problem with the specificity of thiobarbituric acid - malondialdehyde assays as a measure of lipid peroxidation, since this assay does not directly measure the lipid peroxidation reaction [10].

There have been several reports on the decreased antioxidant capacity in asthmatic patients with intense oxidative load. Furthermore, in epithelial lining fluid of asthmatic patients, levels of vitamin E and C having antioxidant capacity were shown to be lower [8]. SOD is an intracellular antioxidant enzyme-inhibiting superoxide anion, which is an important oxidant. In some studies, it is reported that SOD enzyme activity in airway epithelium and erythrocytes of asthmatic patients was lower than that of healthy controls [5, 16]. However, Nadeem et al. [13] have reported that erythrocyte SOD enzyme activity has increased.

On the other hand, in another study, SOD enzyme activity in bronchoalveolar lavage fluid of asthmatic patients was found to be not different from that of

healthy adults. As these patients have mild and stable asthma, it may be thought that pulmonary oxidative stress may not be intense in those patients [2]. In our study, since our patients had mild and stable asthma, erythrocyte SOD enzyme activity might not reveal any statistically significant difference between the groups.

In diseases such as asthma, inflammation may be the most significant contributor to oxidative stress hence the products of oxidative stress are mostly correlated with the activity of the disease [1]. Corticosteroids used in asthma treatment alleviate the oxidative load by inhibiting airway inflammation [6]. Oxidative stress products such as H_2O_2 and NO were found to be increased in expired air of asthmatics. MDA was found to be increased in BAL and the levels of all these three products decreased with corticosteroid therapy [7, 9, 15, 19]. In a study in which the effect of ICS on the erythrocyte SOD enzyme activity was evaluated in asthmatic patients, it was revealed that there is no statistically significant difference between before and after treatment measurements [5].

The information about the effects of regular use of ICS in asthmatic patients on the erythrocyte SOD enzyme activity and plasma MDA level is insufficient. In our study, since the mean plasma MDA level and erythrocyte SOD enzyme activity of asthmatic patients with regular ICS were not significantly different from that of asthmatic patients with no steroid treatment, it can be assumed that these results might have been due to the stability and mildness of our patients and the very few with no ICS.

In this study, we found that the plasma MDA level and erythrocyte SOD enzyme activity are not affected in mild stable asthmatic patients, and ICS treatment does not alter this situation. This finding can be related to either inflammation being mild in stable asthmatics or these parameters not being sensitive enough for inflammation in this group of patients.

References

1. Bowler RP, Crapp JD (2002) Oxidative stress in airways: Is there a role for extracellular superoxide dismutase? *Am J Respir Crit Care Med* 166:s38–43
2. DiSilvestro R, Pacht E, Davis B (1998) BAL fluid contains detectable superoxide dismutase 1 activity. *Chest* 113:401–404
3. Dworski R (2000) Oxidant stress in asthma. *Thorax* 55 (suppl 2):s51–53
4. Emelyanov A, Fedoseev G, Abulimity A (2001) Elevated concentrations of exhaled hydrogen peroxide in asthmatic patients. *Chest* 120:1136–1139
5. Fenech AG, Ellul-Micallef R (1998) Selenium, glutathione peroxidase and superoxide dismutase in maltese asthmatic patients: effect of glucocorticoid administration. *Pulm Pharmacol Ther* 11(4):301–308
6. Hanazawa T, Kharitonov S, Barnes P (2000) Increased nitrotyrosine in exhaled breath condensate of patients with asthma. *Am J Respir Crit Care Med* 162:1273–1276
7. Horvath I, Donnelly L, Kiss A (1998) Combined use of exhaled hydrogen peroxide and nitric oxide in monitoring asthma. *Am J Respir Crit Care Med* 158:1042–1046
8. Kelly F, Mudway I, Blomberg A (1999) Altered lung antioxidant status in patients with mild asthma. *Lancet* 354:482–483
9. Kharitonov SA, Donnelly LA, Montuschi P (2002) Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma. *Thorax* 57:889–896
10. MacNee W (2000) Oxidants/Antioxidants and COPD. *Chest* 117:s303–317

11. McCord JM, Fridovich I (1969) Superoxide dismutase: an enzymatic function for erythrocyte hemoglobin. *J Biol Chem* 244:6049–6055
12. Montuschi P, Corradi M, Ciabattoni G (1999) Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients. *Am J Respir Crit Care Med* 160:216–220
13. Nadeem A, Chhabra S, Masood A (2003) Increased oxidative stress and altered levels of antioxidants in asthma. *J Allergy Clin Immunol* 111:72–78
14. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351–358
15. Ozaras R, Tahan V, Turkmen S (2000) Changes in malondialdehyde levels in bronchoalveolar fluid and serum by the treatment of asthma with inhaled steroid and beta₂-agonist. *Respirology* 5:289–292
16. Raeve H, Thunnissen F, Kaneko T (1997) Decreased Cu, Zn-SOD activity in asthmatic airway epithelium: correction by inhaled corticosteroid in vivo. *Am J Physiol* 272:L148–154
17. Rahman I, Morrison D, Donaldson K (1996) Systemic oxidative stress in asthma, COPD, and smokers. *Am J Respir Crit Care Med* 154:1055–1060
18. Repine J, Bast A, Lankhorst I (1997) Oxidative stress in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 156:341–357.
19. Zanconato S, Scollo M, Zaramella C (2002) Exhaled carbon monoxide levels after a course of oral prednisone in children with asthma exacerbation. *J Allergy Clin Immunol* 109:440–445

Accepted for publication: 23 June 2003