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The Association between Oxidative Stress and Obstructive Lung Impairment in Patients with COPD

Z. KLUCHOVÁ, D. PETRÁŠOVÁ[1], P. JOPPA, Z. DORKOVÁ, R. TKÁČOVÁ

Department of Respiratory Medicine and Tuberculosis, and [1]Department of Experimental Medicine, Faculty of Medicine, P.J. Šafárik University and L. Pasteur Teaching Hospital, Košice, Slovakia

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Summary

An oxidant/antioxidant imbalance is thought to play an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD). We hypothesized that antioxidant capacity reflected by erythrocyte glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) activities, and serum levels of the lipid peroxidation product malondialdehyde (MDA), may be related to the severity of obstructive lung impairment in patients with COPD. Erythrocyte GPx, SOD and CAT activities, and serum levels of MDA were measured in 79 consecutive patients with stable COPD. Pulmonary functional tests were assessed by bodyplethysmography. Moderate COPD (FEV_1 50-80 %) was present in 23, and severe COPD (FEV_1 < 50 %) in 56 patients. Erythrocyte GPx activity was significantly lower, and serum MDA levels were significantly higher in patients with severe COPD compared to patients with moderate COPD (GPx: 43.1±1.5 vs. 47.7±2.9 U/gHb, $p<0.05$, MDA: 2.4±0.1 vs. 2.1±0.1 nmol/ml, $p<0.05$). Linear regression analysis revealed a significant direct relationship between FEV_1 and erythrocyte GPx activity ($r = 0.234$, $p<0.05$), and a significant inverse relationship between FEV_1 and serum MDA levels ($r = -0.239$, $p<0.05$). However, no differences were observed in the erythrocyte SOD and CAT activities between the two groups of patients with different severity of COPD. Findings of the present study suggest that antioxidant capacity reflected by erythrocyte GPx activity and serum levels of the lipid peroxidation product MDA are linked to the severity of COPD.

Key words

Chronic obstructive pulmonary disease • Oxidative stress • Glutathione peroxidase • Lipid peroxidation products • Malondialdehyde

Introduction

COPD represents a major health problem, and its prevalence and mortality rates are increasing worldwide (Pauwels *et al*. 2001). Oxidative stress, defined as an imbalance between increased exposure to oxidant and/or decreased antioxidative capacities, represents one of the key pathogenetic mechanisms in the development of COPD (Repine *et al*. 1997). A number of antioxidant disturbances have been observed in patients with COPD. Lipid peroxidation products, one of the key indicators of oxidative stress (MacNee 2005), are elevated in sputum and exhaled breath condensate of patients with COPD (Tsukagoshi *et al*. 2000, Montuschi *et al.* 2000). Markers

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

of oxidative stress are increased even further during exacerbations of COPD (Dekhuijzen *et al*. 1996), and in patients with very severe form of this disease (Kostikas *et al*. 2003). At the same time, the antioxidant mechanisms are attenuated in these patients, as indicated by reduced glutathione levels in the lungs (Drost *et al*. 2005), reduced glutathione peroxidase (GPx) activity in erythrocytes (Duthie *et al*. 1991) and lower antioxidant capacity in plasma (Rahman and MacNee 2000) during exacerbations of COPD.

Nevertheless, studies on the relationships between the oxidant/antioxidant imbalance and pulmonary functions showed inconsistent results. On the one hand, airway obstruction, reflected by reductions in forced expiratory volume in one second (FEV_1), was shown to correlate with glutathione, myeloperoxidase, and eosinophilic cationic protein levels (Linden *et al.* 1993). In addition, erythrocytes from smokers had decreased GPx activity and were more susceptible to lipid peroxidation than erythrocytes from nonsmokers (Duthie *et al*. 1991). Furthermore, lipid peroxidation products measured as malondialdehyde (MDA) content correlated inversely with the degree of small airway obstruction (Petruzzelli *et al*. 1990). On the other hand, however, more recent studies failed to find a significant relationship between plasma antioxidant capacity and pulmonary function in patients with COPD (Rahman *et al*. 2000). One of the reasons for such discrepancies may be related to differences in the design and study populations across these studies. In addition, Rahman *et al*. (2000) underlined that due to the variability of oxidative stress in smokers it is unlikely that measurements of antioxidant capacity in plasma would correlate closely with the measurements of airway obstruction. However, there is generally less intraindividual variability in the activities of antioxidative enzymes in erythrocytes (Andersen *et al*. 1997). The aim of the present study was to assess the relationships between the severity of COPD and erythrocyte activities of several key antioxidant enzymes, including GPx, superoxide dismutase (SOD), and catalase (CAT). In addition, we characterized the degree of oxidative stress, reflected by MDA levels, in relation to the severity of obstructive lung impairment.

Methods

Patients with COPD were consecutively recruited to the study at the Department for Respiratory Disorders and Tuberculosis at the L. Pasteur Teaching Hospital in Košice, Slovakia. All patients had COPD according to the American Thoracic Society/European Respiratory Society guidelines (Pauwels *et al.* 2001). Exclusion criteria were respiratory disorders other than COPD, malignancy, overt cardiac failure, recent surgery, severe endocrine, hepatic or renal diseases, and use of anticoagulant medication.

Pulmonary functional tests were evaluated with the use of bodyplethysmography (Jaeger, Germany). All pulmonary function testing was performed according to the European Respiratory Society standards with the patients in a sitting position by the same technician in order to ensure consistency of the technique. Three technically acceptable measurements were performed in each patient, and the highest value was included in the analyses.

Arterial blood gases were determined in samples obtained while breathing room air by puncture of the radial artery in the patient seated. Blood for routine biochemical analyses, for the assessment of lipid peroxidation products, and for antioxidant enzymes was withdrawn by venepuncture under standardized conditions. Routine biochemical analyses were performed using standard techniques. Lipid peroxidation was assessed by measuring concentrations of thiobarbituric acid reactive substances (MDA) by spectrophotometry at 535 nm (Yagi 1976). MDA levels are expressed as nanomoles of thiobarbituric acid reactive substances formed per milliliter of plasma. GPx, SOD, and CAT activities were determined in washed red blood cells obtained immediately after sampling from the whole blood anticoagulated with EDTA (Ransel test kit, Randox) based on the methods described by Andersen *et al.* (1997). All values are expressed as units per gram hemoglobin.

Continuous variables are shown as means ± S.E.M. Student's t-test was used for comparison of means between two groups. To assess the relationships between selected variables, linear regression analysis was used.

Results

Seventy-nine patients, 63 men and 16 women, were enrolled in this study. They were generally late middle-aged (mean age 64.8±1.5 years), with the average smoking history of 38.9±5.9 packyears.

Moderate COPD (FEV_1 50-80 %) was present in

Table 1. Demographic data and pulmonary functional tests in patients with different COPD severity.

Variable	Moderate COPD (n=23)	Severe COPD (n=56)	P
Age (years)	62.6±3.7	65.6±1.5	NS
Men/Women	17/6	46/10	NS
Packyears	36.0±10.0	34.8±3.5	NS
Body mass index (kg/m^2)	26.8±1.4	24.2±0.7	NS
FVC (%)	82.1±2.6	61.0±2.0	0.001
FEV_1 (%)	62.7±1.8	35.0±1.1	0.001
FEV_1/FVC (%)	61.1±1.6	46.2±1.4	0.001
$PaCO_2$ (kPa)	5.1±0.1	6.4±0.2	0.001
PaO_2 (kPa)	8.7±0.2	7.8±0.3	NS

Data are means ± S.E.M.

Table 2. Parameters of oxidative stress in patients with different COPD severity.

Variable	Moderate COPD (n=23)	Severe COPD (n=56)	P
MDA (nmol/ml)	2.1±0.1	2.4±0.1	<0.05
GPX (U/gHb)	47.7±2.9	43.1±1.5	<0.05
CAT (U/gHb)	4.4±0.2	4.6±0.2	NS
SOD (U/gHb)	997.4±34.6	1039.3±20.8	NS

Data are means ± S.E.M.

23, and severe COPD (FEV_1 < 50 %) in 56 patients. No differences were found in the demographic data between the two groups (Table 1). FVC, FEV_1, and the ratio of FEV_1/FVC were all significantly lower in patients with severe COPD compared to patients with moderate COPD ($p<0.001$ for all spirometric variables). Examination of arterial blood gases revealed significantly higher $PaCO_2$, and a tendency towards lower PaO_2 in patients with severe compared to moderate COPD ($p<0.001$, $p=0.08$, respectively) (Table 1).

The GPx activity was significantly lower ($p<0.05$), and MDA levels were significantly higher ($p<0.05$) in patients with severe compared to patients with moderate COPD (Table 2). In contrast, no differences were seen between the two groups in the erythrocyte CAT and SOD activities (Table 2). Linear regression analysis revealed a significant direct relationship between FEV_1 and erythrocyte GPx activity ($r = 0.234$, $p<0.05$), and a significant inverse relationship between FEV_1 and plasma MDA levels ($r = -0.239$, $p<0.05$).

Discussion

In the present study, we have demonstrated by studying well-defined patients with moderate to severe COPD, that the activity of GPx in erythrocytes, and plasma MDA levels correlate with disease severity as assessed by FEV_1. Patients with severe COPD have the lowest activity of GPx, and the highest MDA levels.

Considerable evidence now links COPD with increased oxidative stress (Repine *et al*. 1997) and, therefore, the status of antioxidant defense mechanisms assumes paramount importance. One puff of smoke contains 10^{14-16} free radicals (Kinnula and Crapo 2003). In addition, an increased burden of free radicals originates from activated neutrophils, the main inflammatory cells in COPD (Repine *et al*. 1997). Numerous studies have shown that oxidative stress is increased in the lungs of patients with COPD compared to healthy subjects, but also compared to smokers without COPD (MacNee 2005). Lipid peroxidation products are elevated in sputum, exhaled breath condensate

(Tsukagoshi *et al*. 2000, Montuschi *et al*. 2000) and plasma (Dekhuijzen 2004) of patients with stable COPD. Moreover, exacerbations of COPD lead to even further elevations in various markers of oxidative stress (Dekhuijzen *et al*. 1996, Kostikas *et al*. 2003). In addition, the oxidant/antioxidant balance is deteriorated further by the depletion of antioxidant mechanisms. Indeed, deficiencies in both enzymatic and non-enzymatic antioxidative systems were described in patients with COPD (Duthie *et al*. 1991, Rahman *et al*. 2000, Drost *et al*. 2005).

Although an early epidemiological study has shown an association between the oxidant/antioxidant imbalance and lung function in the general population (Chan-Yeung *et al*. 1984), discrepant results were found in further studies regarding the relationship between different markers of oxidative stress and spirometric variables in smokers and patients with COPD. Indeed, several (Petruzelli *et al*. 1990, Duthie *et al*. 1991, Linden *et al*. 1993) but not all (Rahman *et al*. 2000) studies documented that certain markers of oxidative stress may be related to smoking and to the severity of obstructive lung impairment in patients with COPD. In the study of Duthie *et al*. (1991) on the effects of smoking on blood antioxidant status, lower GPx activities were documented in erythrocytes of smokers compared to non-smokers. However, Rahman *et al*. (2000) failed to document any relationship between plasma antioxidant capacity and spirometric variables. Therefore, these authors suggested that antioxidant capacity in plasma would be unlikely related to measurements of airway obstruction, due to high intraindividual variability in oxidative stress in plasma caused by smoking (Rahman *et al*. 2000). However, there seems to be less intraindividual variability in antioxidative enzymes measured in erythrocytes (Andersen *et al*. 1997). Indeed, relationships between antioxidative enzymatic systems and lung function impairment were found in those previous reports studying the antioxidative enzymes in erythrocytes (Duthie *et al*. 1991) but not in plasma (Rahman *et al.* 2000). In agreement, our present study suggests a significant relationship between GPx activity in erythrocytes and pulmonary functions in patients with COPD. These findings extend those of Duthie *et al*. (1991) by indicating that the differences in erythrocyte GPx are associated not only with smoking status but also with the severity of COPD.

In contrast to erythrocyte GPx, no differences were observed in erythrocyte SOD and CAT activities between patients with moderate and severe COPD in the present study. One reason for failing to find a significant relationship between erythrocyte SOD or CAT and pulmonary function may be related to the earlier described phenomenon that various enzymatic systems differ substantially in their responses to smoking-induced increases in oxidative stress (Repine *et al*. 1997). Interestingly, however, GPx was found previously to be much more effective than both CAT and SOD in the protection against oxygen-derived free radicals *in vitro* (Raes *et al*. 1987). Therefore, further clinical studies are needed to clarify the role of different antioxidant enzymatic systems in the protection against oxidative stress.

One of the mechanisms by which oxidants can cause lung injury, is lipid peroxidation. Malondialdehyde is the principal and most studied product of polyunsaturated fatty acid production (Del Rio *et al*. 2005). In the present study, a significant inverse relationship between plasma MDA levels and the degree of obstructive lung impairment reflected by FEV_1 was observed. Previously, a negative correlation between plasma lipid peroxides and lung function was described in non-smokers (Schunemann *et al*. 1997). In addition, lipid peroxidation products measured as MDA content correlated inversely with the time elapsed from the last exposure to cigarette smoke and with the degree of small airway obstruction reflected by low maximal expiratory flow rates in smokers (Petruzzelli *et al*. 1990). Our findings extend these original reports by suggesting that high plasma MDA may be associated with lung function not only in smokers without COPD, but also in patients with severe COPD. These observations indicate that lipid peroxidation is markedly increased in patients with severe COPD, in agreement with previous findings showing elevated levels of other markers of lipid peroxidation such as urinary and plasma concentrations of 8-isoprostane (Pratico *et al*. 1998) and exhaled ethane (Paredi *et al*. 2000) in patients with COPD.

In conclusion, our results indicate that the activity of GPx is reduced, and that lipid peroxidation is more active in patients with more severe COPD suggesting that reductions in the capacity of antioxidative enzymes and increases in toxic lipid peroxidation products might be related to the progression of the disease. Further studies are needed to analyze the pathophysiological mechanisms involved in lung injury related to oxidant/antioxidant imbalance.

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Reprint requests

Ružena Tkáčová, Department of Respiratory Medicine and Tuberculosis, L. Pasteur Faculty Hospital, Rastislavova 43, SK-041 90 Košice, Slovakia. Fax: +421 55615 2664. E-mail: rtkacova@central.medic.upjs.sk