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Activity and function in lung injuries due to sulphur mustard

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Abstract
Aim. Pulmonary complications are known to occur in over half the patients exposed to sulphur mustard. Many studies have focused on the clinical complications, often ignoring the pathogenesis of sulphur mustard. Also, the reasons for the variable severity of lung injuries caused by sulphur mustard are unclear. Hence, the current study was performed to evaluate the correlation between superoxide dismutase (SOD) and catalase (CAT) activity and pulmonary function in patients exposed to sulphur mustard. **Methods.** Our study was a comparative cross-sectional survey. Two hundred and fifty incident survivors were selected from the Sardasht population who were exposed to sulphur mustard in 1987. A control group from non-exposed civilians was also selected. We used a pulmonary function test, and SOD and CAT activity was measured in these groups. **Results.** The mean SOD activity in the healthy control group (70.5 ± 10.8 U ml^{-1}) was higher than in the moderate-to-severe group (67.0 ± 6.1 U ml^{-1}) ($p < 0.001$, one-tail ANOVA, least significant difference (LSD) post hoc). The mean activity in the mild group (72.5 ± 6.9 U ml^{-1}) was no higher than in the healthy control group (70.5 ± 10.8 U ml^{-1}) ($p = 0.095$ one-tail ANOVA, LSD post hoc). The mean CAT activity in the healthy control group (4.9 ± 1.5 U ml^{-1}) was lower than in the moderate-to-severe group (8.0 ± 1.8 U ml^{-1}) ($p < 0.001$, one-tail ANOVA, LSD post hoc) and in the mild group (7.5 ± 1.5 U ml^{-1}) ($p = 0.012$ one-tail ANOVA, LSD post hoc). **Conclusion.** According to our findings, it is reasonable to hypothesize that re-establishment of the activation–inactivation or oxidant–antioxidant balance in favour of the activation and antioxidant balances would be useful as a therapeutic strategy to suppress pathological mechanisms underlying lung injuries.

Keywords: *Sulphur mustard, superoxide dismutase, catalase, lung injury*

Introduction

Many Iranians were exposed to sulphur mustard during the Iraq–Iran war from 1980 to 1987 (UN Security Council 1986). Sulphur mustard exposure in its chronic phase could lead to the development of airway hyper-reactivity, chronic bronchitis, bronchiectasis and lung fibrosis. Previous studies showed that bronchiolitis obliterans is the main clinical–pathological finding in these patients (Emad & Rezaian 1999, Khateri et al. 2003).

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Evidence in similar diseases such as chronic obstructive pulmonary disease (COPD) and asthma suggests that free radical excess and antioxidant deficiency in the lung are related to the severity of airflow limitation and hyperactivity (Comhair & Erzurum 2002).

Antioxidant enzymes crucial for protection of the airway against oxidant stress include superoxide dismutases (SOD) and catalase (CAT) (Comhair et al. 2005). SOD and CAT activity are changed in the oxidant-rich environment of pulmonary disease and during pulmonary disease a further change of these activities occurs with enhanced production of oxygen free radicals by inflammatory cells (Comhair & Erzurum 2002). Moreover, these activities in the lung are related to airway hyper-reactivity and airflow limitation (Comhair & Erzurum 2002).

According to the similarity between lung injuries caused by sulphur mustard and pulmonary disease and the role of free radicals in the progression of pulmonary disease, the current study was performed to measure the levels of SOD and CAT activity and to evaluate the correlation between these activities and pulmonary function in patients exposed to sulphur mustard.

Material and methods

The current study was a comparative cross-sectional survey among subjects who have been exposed to sulphur mustard. Approximately 250 patients from Sardasht in northwest Iran were recruited with respiratory signs and symptoms and chronic lung disease according to clinical and laboratory findings, who had been exposed to sulphur mustard in 1987 (Emad & Rezaian 1999). Documentation of sulphur mustard exposure was based on official certification issued by the Veterans Foundation of Iran. We excluded patients with: (1) any history of smoking; (2) heart failure; (3) lung cancer; and (4) family history or prior diagnosis of asthma, because these conditions would alter the superoxide dismutase activity (Comhair & Erzurum 2002). Sixty healthy, non-smoking individuals with no previous or current exposure to sulphur mustard between the ages of 30 and 60 years from the suburbs of Sardasht constituted the healthy, control group. Ethical approval for this research was obtained from the Ethics Committee of the Baqyiatallah Medical Sciences University.

Patients were asked to complete a checklist of variables including age and gender by a research physician, and pulmonary function tests were performed in the patient group before collecting blood samples. This group was divided into two groups: mild ($n = 140$) and moderate-to-severe ($n = 110$) according to spirometric measurements (NIH/NHLBI 2002).

The activity of SOD was determined according to the method of Paoletti & Mocali (1990). The blood samples were also treated with a mixture of ethanol/chloroform (2:1, v/v) and distilled water to eliminate haemoglobin and red blood cells. For assay, triethanolamine–diethanolamine (0.1 M each)–HCl (1.38%) buffer at pH 7.4 was added into cuvettes, followed by 0.27 mM NADH, 5 mM EDTA, 2.5 mM $MnCl_2$ and 0.1 ml of sample. The samples were read on a Genesys 10 UV spectrophotometer (Sigma-Aldrich) at 340 nm for 5 min to obtain a stable baseline value, then the reaction started with 3.75 mM 2-mercaptoethanol and was read again after 10 min. One unit of activity was defined as the amount of enzyme that inhibits the oxidation of NADH by 50% at 25°C.

CAT activity was measured by a colorimetric method as described by Cohen et al. (1970). Pure ethanol (0.01 ml EtOH ml^{-1}) was added to the supernatant and samples were incubated for 30 min in an ice-water bath. Then, 10% Triton X-100 was added to a final concentration of 1.0%. Catalase from bovine liver was used to generate a standard curve (range 5–100 U). One unit of CAT decomposes 1 mol of H_2O_2 per min at 25°C. Hydrogen peroxide (6 mmol l^{-1}) was added to each sample to initiate the reaction. Sodium phosphate (50 mM at pH 7) was used as a control. After 3 min, ammonium sulphate $[(NH_3)_2SO_4]$ was added to stop the reaction. Potassium permanganate (0.01 M KMnO) was added. The potassium permanganate reacts with the residual H_2O_2 to form a complex that is read at 480 nm with a Genesys 10 UV spectrophotometer.

Statistical analysis was performed with SPSS-13 statistical software using Student's *t*-test, Mann–Whitney U tests and χ^2 test depending on the data; *p*-values of less than 0.05 were considered to be statistically significant.

Results

Table I shows the demographic characteristics of the patients and control groups. Age, height and weight are compared in groups.

The mean SOD activity in the healthy control group (70.5 ± 10.8 U ml^{-1}) was higher than in the moderate-to-severe group (67.0 ± 6.1 U ml^{-1}) ($p < 0.001$, one-way ANOVA, least significant difference (LSD) post hoc). The mean activity in the mild group (72.5 ± 6.9 U ml^{-1}) was no higher than in the healthy control group (70.5 ± 10.8 U ml^{-1}) ($p = 0.095$ one-tail ANOVA, LSD post hoc) as shown in Figure 1A.

The mean CAT activity in the healthy control group (4.9 ± 1.5 U ml^{-1}) was lower than in the moderate-to-severe group (8.0 ± 1.8 U ml^{-1}) ($p < 0.001$, one-tail ANOVA, LSD post hoc) and in the mild group (7.5 ± 1.5 U ml^{-1}) ($p = 0.012$ one-tail ANOVA, LSD post hoc) as shown in Figure 1B.

There was linear correlation between SOD and CAT activity and the spirometric measurements (detailed findings are given in Table II). All spirometric variables have a linear correlation with enzyme activity.

Discussion

In our study, the mean SOD activity in the healthy control group was higher than in the moderate-to-severe group and the mean CAT activity in the healthy control group was lower than in the moderate-to-severe and mild patient groups.

In various pulmonary diseases, it has been observed that the SOD system activity may be increased or decreased (Wu et al. 2000). Previous reports showed that

Table I. Demographic characteristics of the sulphur mustard-exposed patients and control subjects. Age, height and weight are compared in groups using the Student's *t*-test. The data presented are means $\pm$ standard deviations.

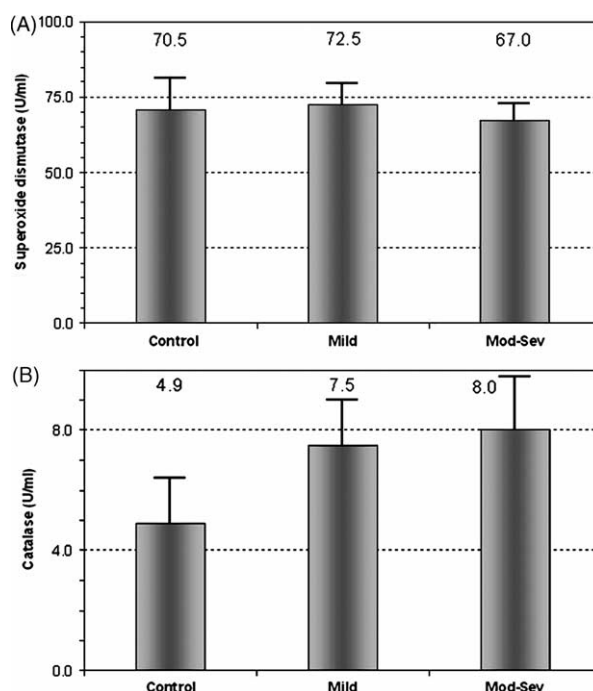|	Chemical group ($n = 250$)	Control group ($n = 60$)	*p*-Value
Age (years)	46.0 ± 10.9	43.14 ± 8.45	0.306
Height (cm)	175.75 ± 86.56	173.03 ± 54.66	0.764
Weight (kg)	71.49 ± 12.31	68.23 ± 15.85	0.287

Figure 1. (A) Superoxide dismutase and (B) catalase activities in the normal, mild and moderate-to-severe patient groups.

localized decreases in SOD activity occurs within asthmatic airway epithelial cells, bronchoalveolar lavage fluid and bronchoalveolar lavage cells in proportion to airflow limitation and asthmatic exacerbations (De Raeve et al. 1997, Mates et al. 1999). Some studies have suggested that SOD might play a role in pulmonary diseases. First, SOD activity is diminished in the cells of bronchoalveolar lavage and brushings from patients with pulmonary disease (Comhair et al. 2000). Second, airway macrophages from individuals with pulmonary disease produce more superoxide than control subjects when stimulated (De Raeve et al. 1997). Third, mice overexpressing SOD are resistant to allergen-induced lung toxicity (Mates et al. 1999), suggesting that extracellular SOD is important in the pathogenesis of pulmonary diseases. However, the role of extracellular SOD in airway diseases such as asthma, COPD and cystic fibrosis is not clear. Animal and human studies have suggested that acute and chronic lung injury may be ameliorated by the administration of antioxidants especially extracellular SOD (MacPherson et al. 2001).

In this study, CAT is the only antioxidant enzyme that was increased. Similarly, in previous studies, CAT increased both at the mRNA and functional levels during human lung morphogenesis (Asikainen et al. 1998). Experiments on human lung have shown that CAT is not elevated by a high oxygen tension in human tracheal epithelial cells during 12 h *in vivo* (Erzurum et al. 1993) or during 48 h *in vitro* (Pietarinen-Runtti et al. 1998). Previous studies on animal models (Engstrom et al. 1990) as well as the present study showed remarkable CAT reactivity, especially in alveolar type II pneumocytes, which are the most resistant cell type in the lung. CAT activity has also

Table II. Correlation between superoxide dismutase (SOD) and catalase (CAT) activity in the plasma and pulmonary function test variables. Data from sulphur mustard-exposed patients are shown.

	Pearson correlation	p-Value
SOD		
VC	0.251	0.001
FVC	0.266	0.001
FEV_1	0.343	0.001
PEF	0.268	0.001
MMEF	0.346	0.001
CAT		
VC	0.327	0.001
FVC	0.343	0.001
FEV_1	0.449	0.001
PEF	0.361	0.001
MMEF	0.361	0.001

VC, vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; PEF, peak expiratory flow; MMEF, maximal middle expiratory flow.

been found to be elevated in senescent fibroblasts, whereas the level of SOD activity remained unchanged (Allen et al. 1999). By contrast, studies by Varshavski et al. (2003) have revealed suppressed activity of CAT in patients with bronchial asthma.

According to our findings, the inflammatory pathways initiated by sulphur mustard may be involved in loss of extracellular SOD activity and an increased CAT activity , perhaps by increasing oxidative stress (Kopff et al. 1994, Varshavskii et al. 2003) and the subsequent inactivation of extracellular SOD. An earlier epidemiological study showed an association between the oxidant/antioxidant imbalance and lung function in the general population (Wooliscroft et al. 1990). Conflicting results were seen in further studies regarding the relationship between different markers of oxidant /antioxidant balance and spirometric variables in smokers and patients with COPD. Indeed, several (Kopff et al. 1994) but not all studies documented that certain markers of oxidative stress may relate to smoking and to the severity of obstructive lung impairment in patients with COPD (Mates et al. 1999).

Further studies are needed to determine the relationship between systemic SOD activity, oxidative modification of proteins and/or glutathione levels to lower airway inflammation. In conclusion, oxidant/antioxidant imbalance in sulphur mustard-exposed patients suffering from pulmonary complications, due to variable changes in extracellular SOD and CAT activity, may lead to progressive inflammation.

Declaration of interest: There is no conflict of interest. The current study has not previously been published elsewhere.

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