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ORIGINAL ARTICLE

Evaluation of activity and phenotype of α1-antitrypsin in a civil population with respiratory complications following exposure to sulfur mustard 20 years ago

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Abstract

Background and aims: The reduced α1-antitrypsin (AAT) activities of some phenotypes have been suggested as contributing to the development of respiratory diseases.

Materials and methods: One hundred patients with respiratory disease following exposure to sulfur mustard were divided into two groups of 50 based on their respiratory symptoms and compared with a healthy control group. AAT phenotypes were determined in the plasma of all patient and control subjects by isoelectric focusing (IEF).

Results: Mean AAT activities in patient and control groups were 3.4 ± 0.3 and 4.2 ± 0.1 μmol min^{-1} ml^{-1}, respectively ($p < 0.001$). No phenotypic alterations were detected.

Conclusions: The difference in the clinical pulmonary symptoms of the two groups was attributed to reduced AAT activity, but this was not manifested as phenotypic changes identifiable by IEF.

Keywords: *Sulfur mustard; α1-antitrypsin; activity; phenotype*

Introduction

Sulfur mustard (SM) exposure in the long term can lead to the development of airway hyper-reactivity, chronic bronchitis and bronchiectasis. However, the pathophysiological basis for the ill health caused by SM needs further study. Currently, there are more than 40 000 people in Iran suffering from pulmonary lesions caused by exposure to mustard gas about 20 years ago (Ghanei et al. 2005). These individuals have been treated for long periods and have been followed up (Shohrati et al. 2007b, Ghanei et al. 2007).

The α1-antitrypsin (AAT), a glycoprotein with a molecular weight of 52 kDa, is a serine protease inhibitor (PI) produced principally in the liver and is found in the blood and epithelial lining fluid of the lungs. AAT is the most abundant antiprotease in the human circulation, and provides more than 90% of the protection against neutrophil elastase in the lower respiratory tract (Stockley 1999, Crystal 1990). It comprises 90% of the total α1-globulin in plasma. The major function of AAT is to inhibit the activity of elastase in the lung, a protease generated by human neutrophil elastase (Carrell et al. 1982, Morse et al. 1977). Under normal conditions, neutrophil elastase is part of a secondary lung defence in response to infection or inflammation (O'Byrne & Postma 1999). However, if not neutralized by AAT, neutrophil elastase can destroy healthy lung tissue. The main effect of PI deficiency is destruction of the pulmonary alveoli resulting in chronic pulmonary disease or emphysema (Crystal 1990, Brantly et al. 1988, Lomas et al. 2004). The connection between PI deficiency and liver diseases in children was first described by Sharp et al. (1969). The PI gene is highly polymorphic with more than 90 different

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alleles described in different populations (Crystal 1989, DeMeo & Silverman 2004).

Various AAT phenotypes and AAT activity have been suggested to contribute to the risk of developing respiratory diseases. Lack of an effective airway protease screen may contribute to the inflammatory process characteristic of respiratory disease, which may lead to an increased risk for the development of respiratory ailments and reduced pulmonary function.

Oxidative mechanisms are also reported to play a crucial role in the genesis of inflammatory lung diseases (Freeman & Transwell 1985, Southorn & Powis 1988, Riley & Kerr 1985). The decreased levels or activity of AAT are attributed in part to post-translational changes induced by oxidative stress (Carp et al. 1982, Taggart et al. 2000).

There are two main mechanisms that can alter AAT function. First, changing activity of AAT and, second, variation in typing. To investigate the role of AAT in respiratory diseases, we studied the possible ways in which it induces respiratory distress in these patients by evaluation of the two mechanisms mentioned above, i.e. modified phenotype and reduced AAT activity, in patients with chronic obstructive pulmonary disease (COPD) following exposure to SM. We evaluated the AAT phenotype and activity in the civil population of Sardasht, a city with about 12 000 population in northwest Iran, almost 20 years after large-scale wartime exposure to SM.

Material and methods

Subjects and blood sampling

A total of 100 patients with respiratory disease who were exposed to SM in 1987 were selected randomly. The documentation of SM exposure was based on the official certification issued by the Veterans (Janbazan) Foundation. Inclusion criteria were documented exposure to SM and diagnosis of chronic pulmonary lesions due to mustard. The following characteristics were considered as exclusion criteria: (1) smoking; (2) voluntary discontinuation of the diagnostic process; (3) heart failure; (4) lung cancer; (5) family history or prior diagnosis of asthma; and (6) pneumonia and/or acute bronchitis. The patients were divided into two groups: mild ($n = 50$) and moderate to severe ($n = 50$), based on the spirometric measurements (forced expiratory volume in 1 s, FEV_1). Complexity of respiratory syndromes, cough, dyspnoea, haemoptysis, sputum and heart burn (gastro-oesophageal reflux disease, GERD) was determined based on the clinical examination and history taking of the patients. Also, 50 healthy non-smokers without any history of exposure to SM from the suburbs of Sardasht were randomly selected and included to form a healthy matched control group. Written informed consent was obtained from all the participants. This study was approved by the ethical committee of the Chemical Injuries Research Center of Baqiyatallah Medical Sciences University.

Blood samples were taken in the morning after 10–12 h fasting between 08:30 and 10:30 a.m. Samples were stored at -70°C before analysis.

AAT phenotyping

AAT phenotypes were determined in all the patients and control subjects by isoelectric focusing (IEF) of blood samples. IEF was performed according to the standard procedure described by Jeppsson & Franzen (1982). Briefly, IEF was performed on a multiphor apparatus (Model LKB 2117; Pharmacia, Piscataway, NJ, USA) connected to a power supply (Model LKB 2103; Pharmacia). Each serum protein electrophoresis (SPE) gel was made to a final concentration of acrylamide 7.5% (w/v), carrier on ampholytes 1% (w/v) with a narrow pH range (4.2–4.9) and glycerol 16% (w/v). Polymerization of the gel was achieved with ammonium persulfate and TEMED. Samples were applied by means of a 8×4 no. 3 filter paper pad (Watman, Clifton, NJ, USA). Prefocusing was performed in 750 V h^{-1} following focusing in 2000 V h^{-1} at a constant current. Fixation, staining and destaining of the gels were carried out. The sample profiles were then compared with standard serum phenotypes. The standard phenotypes were kindly donated by the AAT laboratory, Ullevaal University Hospital, (Oslo, Norway).

AAT activity test

Trypsin inhibitory capacity (TIC) was considered as AAT activity. TIC was assessed using BAPNA (*N*-benzoyl-DL-arginine-*p*-nitroanilide).

Statistical analysis

Statistical analysis was performed by SPSS-13 statistical software using two-way ANOVA; *p*-values of less than 0.05 were considered statistically significant.

Results

Mean age (± SD) of the patients was 46.2 (± 10.9) years. The control group did not differ significantly from the patient group in age, weight, height or gender composition. Demographic characteristics of patients and control groups are shown in Table 1.

Figure 1 shows the densitometric pattern of SPE performed on normal and deficient plasma proteins.

Figure 2 shows a schematic of AAT by IEF in a narrow pH gradient, pH 4.2–4.9. The relationship of TIC and severity of symptoms are shown in Figure 3.

Frequency distribution of respiratory syndromes, cough, dyspnoea, haemoptysis, sputum and heartburn in the patients, derived using a questionnaire, are shown in Table 2. The moderate to severe group had more problems than the mild group in the complexity of respiratory syndromes such as dyspnoea ($p < 0.001$), cough ($p < 0.01$) and haemoptysis ($p < 0.05$). Other symptoms were not significantly different in the two groups although a trend toward more severity in the moderate to severe group was noted.

Mean TIC in the patient group was 3.4 ± 0.3 μmol min^{-1} ml^{-1} which was significantly lower than in the control group (4.2 ± 0.1 μmol min^{-1} ml^{-1}, $p < 0.001$). The phenotype of AAT in the patient and control groups was normal (i.e. MM) and therefore no phenotypical differences were observed in the SM-affected patients compared with the control group.

Table 1. Demographic characteristics of patients and control groups.

Variable	Patients	Control	*p*-Value
Age (years)	46.2 ± 10.9	43.14 ± 8.4	N.S.
Height (cm)	175.7 ± 8.5	173.1 ± 5.6	N.S.
Weight (kg)	79.4 ± 12.3	68.23 ± 15.8	N.S.
Gender (M/F)	70/30	31/19	N.S.

N.S., non significant

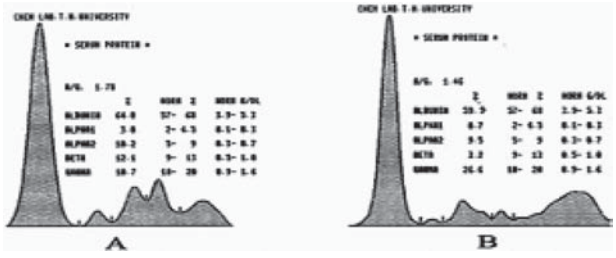

Figure 1. Selected densitometric patterns of serum protein electrophoresis. (A) Normal sample; (B) deficient sample.

Figure 2. A schematic drawing showing α1-antitrypsin by isoelectric focusing in a narrow pH gradient, pH 4.2–4.9.

Discussion

The TIC in these patients was significantly lower than in the normal group. The phenotype of AAT in the patient and control groups was normal (MM) and therefore there was no modified phenotype in this setting.

SM exposure can lead to the development of airway hyper-reactivity, chronic bronchitis and bronchiectasis. The major physiological role of AAT is to protect the supporting elastic tissue in the lung alveoli from hydrolysis by neutrophil elastase which ultimately predisposes the individuals to the development of emphysema, infections leading to respiratory complications and bronchiectasis (Taggart et al. 2000, Miravitlles et al. 1999). A study database demonstrated that AAT deficiency is one of the most serious hereditary disorders affecting individuals in all racial subgroups worldwide (de Serres 2002). There are a number of case reports describing patients with primary immune deficiency and AAT deficiency (Ostergaard 1984, Gelfand et al. 1979, Phung et al.

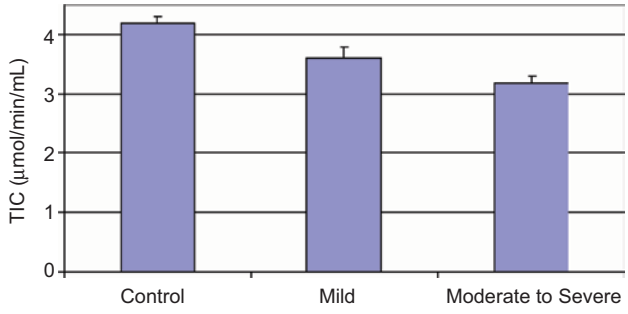

Figure 3. The α1-antitrypsin activity measured in the patients (mild and mild to severe groups) and the control group. (The distribution of patients into the three groups was done before the trypsin inhibitory capacity (TIC) assay.)

Table 2. Dyspnoea, cough, sputum, haemoptysis frequency and heartburn frequency in the patients, based on questionnaires.

Symptom	Scale	Subjects, *n*
Dyspnoea	In extraordinary exercises	18
	In ordinary exercises	60
	In mild exercises	22
Cough	Without serious problem	30
	Sometimes disturbs work	60
	Usually disturbs work	10
Sputum	Never	0
	Before	14
	Current	86
Haemoptysis	Never	54
	Before	46
	Current	0
Heartburn (GERD)	Never	0
	Before	84
	Current	16

GERD, gastro-oesophageal reflux disease.

1982, 1983). There is also some evidence relating AAT deficiency to primary antibody deficiencies (Sansom et al. 2002, Gedde-Dahl et al. 1981) and occurrence of pulmonary diseases (emphysema and bronchiectasis) (O'Byrne & Postma 1999). Shohrati et al. (2007a) showed that equilibrium of the oxidant/antioxidant component is very important for living organs and disequilibrium (oxidant excess or lack of antioxidant) can cause oxidative stress and thereby pulmonary diseases. They noted that superoxide dismutase (SOD) and catalase (CAT) activity was reduced in SM-affected patients compared with healthy subjects (the same subjects as in the present study). Free radicals are one of the causes of this imbalance toward oxidation. They also showed that oxidation imbalance due to oxidative stress can occur in SM-affected subjects. The authors evaluated the effect of *N*-acetyl cysteine as an antioxidant in a controlled clinical trial study (Shorati et al. 2008). The results showed that long-term prescription of *N*-acetyl cysteine was significantly effective in improving respiratory complications in patients with COPD caused by exposure to SM. This is in concordance with evidence showing the effectiveness of antioxidants in preventing SM-induced oxidative stress in cellular models (Rappeneau et al. 2000, Atkins et al. 2000). Therefore, the differences in the clinical symptoms in our patients compared with the control group can be attributed to diminished AAT activity due to oxidative stress in the process of disease following exposure to SM. No significant difference in PI phenotype was noted and therefore an effect of SM on the mutation of the AAT gene is unlikely. It could be concluded that just a single exposure to SM has no effect on changing the phenotype of AAT. Our findings are in concordance with those of Fazlollahi et al. (2006) who also found no statistically significant differences in the proportion of AAT phenotype in a population with bronchiectasis and a matched control group. A complementary study on a larger population would be desirable to confirm the lack of effect of SM on AAT phenotype. A further *in vitro* study is on the way to confirm the effect of oxidative stress in AAT deactivation in SM-exposed patients.

Unfortunately, a pre-existing deficit in AAT activity in some sulfur mustard-exposed patients that might predispose them to respiratory disease could not be ruled out by this study. Furthermore, a decrease of AAT activity is related to underlying pathology of COPD in this setting and we could not consider it as direct effect of exposure to SM after 20 years.

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