International Immunopharmacology 17 (2013) 958–963

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Chemokines, MMP-9 and PMN elastase in spontaneous sputum of sulfur mustard exposed civilians: Sardasht-Iran Cohort Study

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ARTICLE INFO

Article history:
Received 12 December 2011
Received in revised form 6 December 2012
Accepted 27 December 2012
Available online 28 January 2013

Keywords:
Sulfur mustard
Chemokines
MMP-9
PMN elastase
Sputum
Sardasht-Iran

ABSTRACT

Chemokines play an important role in acute and chronic pulmonary diseases. The aim of this study was to evaluate the levels of chemokines, MMP-9, and PMN elastase in spontaneous sputum and serum of patients 20 years after SM exposure. In context of Sardasht-Iran Cohort Study (SICS) 40 male volunteers with a history of SM exposure in June 1987 and complain of excessive sputum were recruited. The volunteers were clinically examined and their history was collected by internists. Sputum and serum levels of IL-8, fractalkine, MCP-1, RANTES, MMP-9, and PMN elastase were measured using ELISA kits (R&D System). Spirometries were performed on all the participants. Sputum level of fractalkine was significantly lower in the hospitalized group (N = 16, Median = 1.05; IQR = 0.41–2.62) than non-hospitalized group (N = 18, 4.031; IQR = 0.947–8.203) ($p = 0.042$). However, serum levels of fractalkine were higher in the hospitalized group (Mean ± SD = 2.08 ± 5.09) than in the non-hospitalized (Mean ± SD = 0.53 ± 0.87) group (T-test, $p = 0.03$). Serum levels of PMN-elastase were also higher in the hospitalized group (Mean ± SD; 64,794.43 ± 26,820.08) than in the non-hospitalized group (Mean ± SD = 44,049.33 ± 17,675.85) ($p = 0.017$). There was no relationship between the cytokines and the studied factors in sputum and the GOLD classification, but the serum levels of fractalkine and MMP-9 were significantly higher in the more severe (grades 3–4) group. There was no significant correlation between sputum and serum levels of measured inflammatory mediators and pulmonary complications in the patients who were exposed to SM 20 years earlier. Pathophysiologic process involved in SM induced pulmonary problems might be different from those in other chronic pulmonary diseases such as COPD and asthma.

1. Introduction

The lungs, eyes and skin are the organs mostly affected by SM toxicity [1,2]. Of these, the lungs are the site of the most common and most disabling long-term complications. The mechanism(s) of mustard gas toxicity is not well known. To understand more its mechanisms, a comprehensive historical cohort study was recently established in a civilian SM-exposed population in Sardasht, Iran which is defined as the Sardasht-Iran Cohort Study (SICS) [3]. In these patients, dyspnea was the most common symptom. Chronic cough, sputum, hemoptysis, and chest pain were also common pulmonary findings in this population [4]. A previous study by the same group showed that the serum levels of pro-inflammatory cytokines IL-1a, IL-1b, IL-1Ra and TNF were significantly lower in the SM-exposed group [5]. However, the serum levels of MCP1/CCL2

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http://dx.doi.org/10.1016/j.intimp.2012.12.015

were significantly higher and the level of MMP9 and fractalkine showed no change in the cohort [6].

In general terms, chemokines have been shown to play an important role in acute and chronic pulmonary diseases [7]. IL-8/CXCL8 is a chemokine secreted from various sources in response to different stimuli and participate in, acute inflammation due to potent actions on neutrophils. IL-8/CXCL8 plays a role in the pathogenesis of chronic pulmonary diseases such as COPD, pulmonary fibrosis and asthma [8]. RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted) is involved in inducing fibrous airway obliteration (FAO) in transplanted lungs and as such local administration of anti-RANTES might be a therapeutic option for bronchiolitis obliterans (BO) following lung transplantation [9]. Monocyte chemoattractant protein-1 (MCP-1) belongs to the C–C chemotactic cytokine family and plays certain role in the pathogenesis of fibrotic lung diseases. MCP-1 recruits monocytes, memory T cells, and dendritic cells to the site of tissue injury, infection, and inflammation. Increased expression of MCP-1 in the lung tissues of patients with idiopathic pulmonary fibrosis (IPF) has been reported [10]. Fractalkine (CX3CL1) is the only member of the CX3C chemokine family which exists as a membrane-bound and soluble form and interacts with cells expressing its specific receptor (CX3CR1). Fractalkine has chemotactic, adhesive, and cytotoxic activities. It has been shown that this chemokine plays certain roles in the pathogenesis of various lung diseases [11]. Matrix metalloproteinase-9 (MMP-9) is responsible for degradation of extracellular matrix (ECM) components including basement membrane collagen. Low levels of MMP-9 have been reported in normal lungs but it has been found to increase significantly in several lung diseases including asthma, idiopathic pulmonary fibrosis (IPF), and chronic obstructive pulmonary disease (COPD). Specific tissue inhibitors of matrix metalloproteinase (TIMP) regulate the potent proteolytic activities of MMPs [12,13]. Neutrophil elastase is a protease which is involved in tissue destruction and inflammation and plays an important role in the pathogenesis of various lung diseases including emphysema, chronic obstructive pulmonary disease, cystic fibrosis, and adult respiratory distress syndrome [14,15].

A few studies have evaluated the role of chemokines in SM-related pulmonary complications. Emad et al. reported the elevated levels of IL-8, IL-1b, IL-6, TNF-a, and IL-12 in the bronchoalveolar lavage fluid (BALF) of patients with SM-induced pulmonary fibrosis [16,17]. Previous studies of the same group of SICS also showed that the serum levels of IL-8, IL-6 and RANTES/CCL5 were significantly decreased in the SM-exposed group. No significant difference was observed in the serum levels of MCP-1/CCL2 or fractalkine/CX3CL1 between the patients with SM-induced pulmonary complications and the control groups. No significant association was seen between the serum levels of IL-8 and IL-6 and pulmonary symptoms, signs, and spirometry parameters, but the serum levels of RANTES/CCL5 were significantly decreased with lower FEV1/FVC [6,18]. Since local levels of chemokines, MMPs, and other inflammatory mediators are more important than circulatory levels in pulmonary disease, and the sputum collection is less-invasive than bronchoscopy for BALF, in the current study the sputum levels of IL-8/CXCL8, RANTES, MCP-1, fractalkine, MMP-9, and PMN-elastase were evaluated and compared with the serum levels. The relationship between the inflammatory mediators and pulmonary symptoms, signs and function was assessed.

2. Material and methods

2.1. Participants

The participants in the present study were a subgroup of 40 male SM-exposed individuals in the Sardasht-Iran Cohort Study (SICS). Based on the medical record documents which are verified by the Medical Committee of the Foundation of Martyr and Veterans Affairs, the participants have been exposed to SM in 1987. The volunteers had spontaneous sputum without any clinical symptom of acute infection or febrile condition. Patients with history of immunosuppressive drug consumption in the past month or history of chronic inflammatory diseases were excluded. Other criteria for patient selection have been previously reported in the SICS methods [3].

Participants were classified into two groups; hospitalized and non-hospitalized based on the hospitalization and severity of the clinical problems at the time of exposure. The investigators used hospitalization as an index for the severity of exposure and pulmonary involvement. The age range of the participants was 20–60 years (Mean of 44.3 ± 9.8) for all the participants and 46.9 ± 7.6 for hospitalized vs. 41.7 ± 11.3 years for non-hospitalized, there was no significant difference between the two groups (Chi square = 0.111).

2.2. Ethical considerations

The study was approved by the ethical committee of the Board of Research Ethics of Janbazan Medical and Engineering Research Center (JMERC), the Board of Research of Ministry of Health, and Shahed University. Individuals who wish to participate and sign an informed consent were recruited.

2.3. Clinical evaluation

Internists examined the study participants and completed a questionnaire surveying pulmonary symptoms (chronic cough, sputum, hemoptysis, and dyspnea) and pulmonary findings (fine crackles, coarse crackles, and wheezing). Chronic cough was defined as persisting cough for more than 3 weeks. Three subsequent spirometry measurements (Chest 801 Spirometry) were performed on all participants according to the American Thoracic Society Criteria under supervision of a trained nurse. The suitable measurement was selected for data collection. The classification of severity of pulmonary involvement was done according to the Global initiative for chronic Obstructive Lung Disease (GOLD).

2.4. Sputum collection and processing

Participants were instructed to cough sputum into a sterile container if they felt that sputum might be present. After the removal of the saliva, the specimen was weighed and a 4-fold volume of phosphate buffered saline (PBS) was added to disperse the sputum. After 10 min of vortex, each sample was centrifuged, aliquoted, and kept frozen at −70 °C for later cytokine analysis.

2.5. Serum collection

Peripheral blood sample was drawn into Vacutainer tubes (BD Biosciences). The serum was separated for 20 min by centrifugation at 2000 ×*g* (4 °C), aliquoted, labeled and kept frozen at −80 °C until laboratory measurements.

Table 1
Comparison of clinical findings between hospitalized and non-hospitalized groups exposed to sulfur mustard in 1987 Sardasht, Iran.

Clinical findings	Hospitalized (n = 20)		Non-hospitalized (n = 20)		p-Value
	n	Percent	n	percent	
Chronic cough	18	90	18	90	0.999
Hemoptysis	4	20	8	40	0.168
Dyspnea	19	95	16	80	0.342
Fine crackles	1	5	5	25	0.064
Wheezing	4	20	5	25	0.282
Coarse crackles	0	0	2	10	0.947

Clinical findings were not significantly different in the two groups at the time of study (twenty years after exposure). Chi square with exact Fisher test.