Systemic Inflammation in Chemical Lung Veterans with Mustard Gas

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ABSTRACT

Background and Objective: Chronic obstructive pulmonary disease (COPD) can be defined as a “a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases. The effects of sulfur mustard poisoning may be local, systemic, or both, depending on environmental conditions, exposed organs, and extent and duration of exposure. In this paper we discuss about Systemic Inflammation factors in Chemical Lung Veterans with Mustard Gas (Mustard Lung) and Patients with Chronic Obstructive Pulmonary Disease (COPD). We also compare these factors between two mentioned groups.

Material and Methods:

Results:

Conclusion: In COPD patients due to SM exposure inflammatory markers (highly sensitive CRP, interleukin 6) are elevated and these markers have direct association with the severity of disease. The finding which recommends the role of systemic inflammation in the pathogenesis of COPD due to SM intoxication like the COPD due to other causes.

The studies discussed in the present review clearly support the concept that chronic obstructive pulmonary disease can no longer be considered a disease affecting the lungs alone. The available evidence indicates that: 1) chronic obstructive pulmonary disease has an important systemic component; 2) clinical assessment of chronic obstructive pulmonary disease ought to take into consideration the systemic components of the disease; and the treatment of these extrapulmonary effects appears to be important in the clinical management of the disease. A greater understanding of the processes involved will allow treatment to be guided with greater precision, rather than relying on bronchodilating agents from which many patients derive minimal benefit. high level of serum CRP is associated with an increased risk of mortality in COPD patients.

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1 Introduction

Sulfur mustard (SM) more commonly called “mustard gas” is one of a class of vesicant chemical warfare agents with the ability to form vesicles or blisters on the exposed skin. SM induces early and late disabling effects on health status [1]. The most important target organs affected by acute toxicity of SM are the respiratory and gastrointestinal tracts, eyes, skin, bone marrow and the immune and central nervous systems [2]. The severity of damage depends largely on the dose and duration of exposure.

Many years after exposure, people who have been exposed to SM are still suffering from its late complications including ocular, coetaneous, respiratory and psychological disorders [3,4]. The effects of sulfur mustard poisoning may be local, systemic, or both, depending on environmental conditions, exposed organs, and extent and duration of exposure.
exposure. Because of the high lipid solubility, sulfur mustard quickly penetrates the lipid cell membrane. Although sulfur mustard may be lethal, it is more likely to cause extensive incapacitating injuries to the eyes, skin, and respiratory tract of exposed persons. Alkylation reactions (replacement of a hydrogen atom in an organic compound by an alkyl group [CnH2n+1]) of sulfur mustard with tissue are rapid and irreversible; however, there is a latency period before effects become apparent. Eye and cutaneous lesions do not become apparent for 30 minutes to several hours after exposure. Burns caused by sulfur mustard may require long healing periods. Local effects are manifested at concentrations/doses far lower than those that produce systemic effects.

According to the definition of the European Respiratory Society (ERS), chronic obstructive pulmonary disease (COPD) is a disorder characterised by reduced maximum expiratory flow and slow forced emptying of the lungs due to varying combinations of diseases of the airways and emphysema [5].

Chronic obstructive pulmonary disease (COPD) can be defined as a “a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases [6].” Current global predictions are that mortality from COPD is set to increase rapidly over the next decade [7].with the consequent burden in terms of direct and indirect costs worldwide likely to be significant.

COPD is a complex inflammatory disease with both airway and parenchymal lung injury. Emphysema and small airway inflammation and damage leads to the enlargement of alveolar air spaces, airway wall fibrosis, loss of elastic recoil, smooth muscle hypertrophy, goblet cell hyperplasia and mucus plugging.

Inflammatory cytokines such as TNF and IL-1 play a pivotal role in coordinating defense response to inflammation through interaction with their receptors. Tumor necrosis factor (TNF) is an important inflammatory cytokine in both acute and chronic inflammations which causes an inflammatory response through interaction with its receptors expressed on various cells such as endothelial cells. These cytokines increase vascular permeability allowing leukocyte access to the site of infection. In addition to this localized inflammatory response, systemic release of TNF may lead to septic shock and death [14]. Although interleukin-1 (IL-1) and TNF are structurally distinct and bind to different receptors, they have many closely related activities. IL-1 is responsible for many changes associated with the onset of a number of medical conditions and is involved in both acute-phase responses and chronic inflammatory conditions. IL-1α and IL-1β are two structurally related forms of IL-1 which bind to two types of receptors present on a variety of target cells. IL-1 affects nearly every cell type, often in concert with other cytokines or other mediators and plays a role in development of disease and also in normal homeostasis [15,16].

The origin of systemic inflammation in COPD is still under debate. Systemic inflammation is considered a hallmark of COPD and one of the key mechanisms that may be responsible for the increased rate of comorbidities, including and osteoporosis [7].

Systemic inflammation may be directly linked to a number of complications commonly encountered in patients with COPD such as cachexia, skeletal muscle dysfunction, depression, and osteoporosis [17]. Levels of Interleukin-6 (IL-6) and C-creative protein (CRP) indicating systemic inflammation are known to be elevated in chronic diseases including chronic obstructive pulmonary disease (COPD) and depression.

Comorbid depression is common in patients with COPD, but no studies have investigated whether proinflammatory...
cytokines mediate the association between pulmonary function and depressive symptoms in healthy individuals with no known history of obstructive pulmonary diseases.

In this paper we discuss about Systemic Inflammation factors in Chemical Lung Veterans with Mustard Gas (Mustard Lung) and Patients with Chronic Obstructive Pulmonary Disease (COPD). We also compare these factors between two mentioned groups.

2 Inflammatory Factor Changes

2.1 In Chemical Lung Veterans with Mustard Gas (Mustard Lung)

The analysis of inflammatory mediators over time indicates that the SM injury progresses to the posterior part of the cornea, initiating the production of cytokines/chemokines, which, in turn, may activate MMPs. MMP-2 and MMP-9 degrade collagen, thus contributing to the deterioration of the stroma.61 It remains to be determined whether SM-damaged corneal stroma might elicit alloreactive responses that lead to further degradation, similar to Mooren’s ulcers. Abnormalities in the immune system may contribute to recurrent infections, septicemia, and a higher incidence of malignancies in these patients.[12]

The most common late complications of sulfur mustard poisoning are respiratory problems, including chronic obstructive pulmonary disease (COPD), chronic bronchitis, bronchiolitis obliterans, bronchiectasis, airway hyperreactivity, and lung fibrosis.[4–8] COPD is a condition characterized by poorly reversible airflow limitation that is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious agents.9 It is now well documented that the inflammatory response in COPD is not limited to the lungs, and also that systemic inflammation plays an important role in its presentation. Inflammatory markers, such as C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor alpha, are also important in the pathogenesis of COPD, and raised levels of these factors have been found in stable COPD patients.[10–12] IL-6 as a proinflammatory cytokine may play a considerable role in the systemic inflammatory response in COPD. [13,14] Several different cell types can produce IL-6, but the main sources are monocytes, macrophages, T and B cells, fibroblasts, epithelial cells, and the smooth muscle cells of the airways. [14] Tumor necrosis factor (TNF) is an important inflammatory cytokine in both acute and chronic inflammations which causes an inflammatory response through interaction with its receptors expressed on various cells such as endothelial cells. These cytokines increase vascular permeability allowing leukocyte access to the site of infection. In addition to this localized inflammatory response, systemic release of TNF may lead to septic shock and death [14]. Although interleukin-1 (IL-1) and TNF are structurally distinct and bind to different receptors, they have many closely related activities. IL-1 is responsible for many changes associated with the onset of a number of medical conditions and is involved in both acute-phase responses and chronic inflammatory conditions. IL-1α and IL-1β are two structurally related forms of IL-1 which bind to two types of receptors present on a variety of target cells. The type I receptor functions for signaling and type II as decoy, in addition to the soluble forms of receptors. IL-1 affects nearly every cell type, often in concert with other cytokines or other mediators and plays a role in development of disease and also in normal homeostasis [15,16].

During inflammation, injury, immunological challenge or infection, IL-1 is produced and contributes to the inflammatory response which may affect both acute and chronic diseases [17,18]. The serum levels of inflammatory cytokines including IL-1α, TNF-α, IL-1β
and IL-6 in the exposed participants with ocular surface problems were significantly lower than their counterpart in the control group, and in those exposed participants without ocular surface problems IL-1α, TNF-α and IL-6 were significantly lower than their counterpart in the control group. Conversely, the serum titers of the CRP and RF in the exposed participants without ocular problems were significantly higher than the control participants without ocular problems. IL-1 is one of the essential factors in wound-healing [22]. There were not any significant differences between the serum levels of MMP-9 in the exposed and control groups with or without ocular problems. There has been no other report on the serum levels of IL-1α, TNF-α, IL-1β, IL-1Ra, MMP-9, IL-6, CRP and RF especially in association with their ocular surface problems in mustard gas exposed patients. Many studies have undertaken the evaluation of local immune responses and detection of the cytokine levels in the eye as the target organ of inflammations in other clinical complications [24,25]. However, it seems that parallel systemic immunological responses have been less considered. In some instances local immunological responses are parallel and in relation with systemic immunological responses or vice versa [26,27].

In the case of MMP-9, there is no study to evaluate the serum levels of MMPs in patients with delayed complications of SM. Some in vitro and animal model studies have reported the association of acute SM complications with changes in MMP expression [29–31]. In a study on mice ear skin 7 days after exposure to SM, relative levels of MMP-9 mRNA and protein were increased 27- and 9-folds respectively compared to the control group [31]. Based on the evidences on the role of MMP-9 in inflammation and neovascularization, it was expected that local and circulating MMP-9 in the SM exposed people with ocular complications are altered, but our data in this study revealed at least the serum level of MMP-9 does not display any association with ocular complications in SM participants. To clarify the role of MMP-9 in pathogenesis of long term ocular complications in SM exposure, it is necessary to assess this inflammatory marker in local samples of eye including tear or ocular tissues.

It is likely that the MMP-9 activation influences the alveolar level, since the enzyme is rapidly inactivated and so measurement of sputum levels of this enzyme might not be accurate. The results of this study suggest no chemokine-induced inflammatory process exists in the sputum and serum of patients 20 years after exposure to SM.

Higher levels of systemic inflammatory markers including CRP and IL-6 are associated with progression of some ocular disease such as age-related macular degeneration (AMD) [35,36], retinal venular diameter changes [37] and even in diabetic retinopathy [38,39]. This is true in the case of the Rheumatoid Factor (RF) in the juvenile chronic arthritis with ocular involvement [40]. In this study the serum levels of IL-6 in the exposed with or without Slit lamp findings were significantly lower than the normal similar controls, on the contrary the serum titers of CRP and RF were significantly higher in the exposed group. Another immunological mechanism may be implicated in this regard.

Increased levels of IL-8 in the bronchoalveolar lavage fluid of patients with sulfur mustard poisoning and late pulmonary complications have been demonstrated.[18] Also, serum levels of highly sensitive C-reactive protein (hs-CRP), as an inflammatory marker, are elevated in patients with sulfur mustard poisoning and are correlated inversely with FEV1.[19] Despite these studies, Pourfarzam et al believed that inflammatory mediators probably do not have any major role in the pathogenesis and persistence of pulmonary complications of sulfur mustard exposure. [20]
2.2 In Chronic Obstructive Pulmonary Disease (COPD) Patients

The inflammatory reaction in the lungs is often associated with systemic inflammation.[34] The systemic inflammatory response is characterized by elevated plasma levels of interleukin (IL)-6, C-reactive protein (CRP), fibrinogen, and surfactant protein D. [15,35] It is postulated that systemic inflammatory response in COPD is largely driven by L-6, which is overexpressed in the lungs of COPD patients and translocates into the systemic circulation, inducing the liver to produce acute phase (inflammatory) proteins such as CRP and fibrinogen and the bone marrow to release leukocytes (e.g., neutrophils, band cells, and monocytes) into the systemic circulation.36 These inflammatory cells are then recruited to sites of active inflammation such as atherosclerotic plaques, where they release myeloperoxidase and other oxidative stress mediators that modify various proteins (e.g., carbamylation and nitrosylation). Some of these modified proteins may becomenew ligands for receptors expressed on foam cells and smooth muscle cells in plaques, causing increased buildup of lipids and activation of plaques. Acute (on chronic) inflammation may cause acute atherothrombosis by destabilizing vulnerable plaques and inducing a prothrombotic state. Interestingly, systemic inflammatory mediators in COPD (such as CRP, fibrinogen, surfactant protein D, and neutrophils) have been associated with increased risk of CVD morbidity and mortality and thus may serve as biomarkers of this process. [35,37,38]

Numerous studies have reported increased levels of circulating cytokines and acute phase reactants in the peripheral circulation of patients with COPD [39–43]. Abnormalities include increased concentrations of TNF-a, its receptors (TNFR-55 and TNFR-75), IL-6, IL-8, C-reactive protein, lipopolysaccharide-binding protein, Fas and Fas ligand [39–43]. These abnormalities were seen in patients considered clinically stable, but were generally more pronounced during exacerbations of the disease [42]. Some authors have shown that peripheral monocytes harvested from patients with COPD are capable of producing more TNF-a when stimulated in vitro than those obtained from healthy controls [45]. This was particularly evident in patients with COPD and low body weight, suggesting that excessive production of TNF-a by peripheral monocytes may play a role in the pathogenesis of weight loss in COPD (see Nutritional abnormalities and weight loss section) [45].

Increased systemic inflammation during acute exacerbations of COPD is indicated by increased levels of the acute phase proteins C-reactive protein (CRP) and fibrinogen, elevated levels of cytokines as IL-6, and the neutrophil marker MPO.[9–11] In addition, the anti-inflammatory mediator sIL-1RII was shown to increase progressively during treatment of exacerbation. Recently, Hurst et al. published an extensive evaluation of a panel of potential biomarkers at exacerbation, from which CRP was the most selective biomarker.[13] Lung epithelial cells have been shown to release mediators after exposure to oxidative stress that may play an important role in the development of COPD. For example, Transforming Growth Factor-β1 (TGF-β1) expression is increased in small airway epithelial cells of smokers compared to nonsmokers and even more increased in patients with COPD. TGF β mRNA levels correlated positively with the extent of smoking history and the degree of airway obstruction [42,43]. In addition, the exposure of primary human bronchial epithelial cells cultured from surgical tissue to 400 ppb NO2 results in the release of IL-8, TNFα, and GM-CSF [44], cytokines known to be increased in COPD. Interestingly, TGF- β1 has been suggested to increase oxidative stress [45,46]. In vitro studies have demonstrated that 4-HNE, as
present in lung tissue in COPD patients, increased TGF-β1 expression by a mechanism dependent on the activation of AP-1 in macrophages [47]. Furthermore, TGF-β1 causes a marked decrease in glutathion levels in endothelial and epithelial cells and a downregulation of gamma-glutamylcysteine synthetase (Gamma-GCS) mRNA levels in alveolar epithelial cells [48].

TNFα is a proinflammatory cytokine produced by endothelial cells and macrophages which causes upregulation of other cytokines. In severe COPD, TNFα activates neutrophils, macrophages and epithelial cells, causes MMP release from macrophages and inhibits skeletal muscle protein expression, thereby contributing to the loss of body mass seen in COPD (hence its previous name - cachexin) [17, 25].

Cytokines and chemokines also play an important role in cellular recruitment and the pathophysiology of COPD with interleukin-8 (IL-8), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNFα) likely to be important. A correlation between sputum IL-8 titres and severity of airflow obstruction has been reported [15]. Another neutrophil chemoattractant, Leukotriene B4, is also increased in induced sputum of COPD patients [17].

Like IL-8, IL-6 is produced by macrophages and epithelial cells [25]. In one study, both IL-6 and IL-8 were noted to be present in higher levels in sputum in patients with frequent exacerbations, especially IL-6, with a further rise during an exacerbation [26]. There may be a correlation between IL-6 levels and years of cigarette smoking and in the absence of increased levels of neutrophils and macrophages, the authors hypothesised that the rise in IL-6 was from bronchial epithelial cells. The precise role of IL-6 in COPD is unknown. IL-6 is known to induce lipolysis, suppress TNFα production and stimulate the production of cortisol [27]. The IL-6 gene is activated in contracting skeletal muscle, in particular when muscle glycogen stores are low, thereby causing an increase in circulating IL-6 levels during exercise [28]. Due to the suppression of TNFα production by IL-6, an anti-inflammatory effect due to exercise mediated by this cytokine has been hypothesised [27].

MMPs are family of enzymes, each with individual substrate specificity, which together are thought to have the potential to degrade most of the proteins in the extracellular matrix [16, 29]. MMP-8 and MMP-9 (a gelatinase and a collagenase respectively) were found to be in a greater net state of activation in a significant number of patients with COPD compared with a smaller proportion of healthy smokers [29]. This study also found correlation between MMP-8 and MMP-9 activation and absolute numbers of neutrophils.

3 Comparison Between Inflammatory Factors in Mustard Lung and COPD Patients

In the SM-exposed patients, the serum pro-inflammatory cytokine levels were significantly lower in the exposed group than in controls. There was also significant positive correlation between concentration of all of mentioned cytokines, the strongest being between IL-1β and TNF.

In contrast to our expectation, CXCL8/IL-8 showed significant decrease in the sera of the exposed cases compared to the control. Regarding the elevated levels of CXCL8/IL-8 in other chronic inflammatory disorders including COPD, BO, bronchitis and asthma and concerning to chronic inflammatory disorders in SM intoxicated patients [4,23,29,40–44], it is suggested that different pathophysiological and molecular mechanisms of immune and inflammatory processes are involved in SM pulmonary disorders. In addition this might be due to the presence of auto-antibodies
against CXCL8/IL-8 as their presence has serum levels of IL-6 are raised in chemical warfare veterans with pulmonary complications due to sulfur mustard exposure, and this inflammatory marker has a direct correlation with severity of airways disease.

Even with the exclusion of smoking, as well as inflammatory and systemic disorders, serum levels of IL-6 were significantly higher in patients than in controls. This finding provides evidence of the possibility of an inflammatory basis for the late pulmonary complications of sulfur mustard exposure and is in accordance with previous studies in other COPD patients which pointed out that, even during the stable phase of COPD, serum levels of inflammatory markers, including IL-6, may be raised.

Previous research in COPD patients has revealed that the serum IL-6 level is an independent predictor of exercise tolerance. Serum levels of IL-6 are raised in chemical warfare veterans with pulmonary complications due to sulfur mustard exposure, and this inflammatory marker has a direct correlation with severity of airways disease.

Increased levels of IL-8 in the bronchoalveolar lavage fluid of patients with sulfur mustard poisoning and late pulmonary complications have been demonstrated. Serum levels of highly sensitive C-reactive protein (hs-CRP), as an inflammatory marker, are elevated in patients with sulfur mustard poisoning and are correlated inversely with FEV1. In a study by Sin et al inhaled corticosteroids alone or in combination with a beta-agonist did not reduce serum CRP and IL-6 levels in patients with moderate to severe COPD, but did decrease serum surfactant-D protein levels.

The authors reported elevated C-reactive protein (CRP), fibrinogen, leucocytes and TNFa levels in COPD patients compared to healthy subjects. They also provided evidence that persisting low grade inflammation was present in ex-smokers with chronic airflow limitation. The same group have extended their findings in a subsequent study to report that elevated CRP levels may provide prognostic information in terms of adverse clinical outcome.

Cytokines and chemokines also play an important role in cellular recruitment and the pathophysiology of COPD with interleukin-8 (IL-8), interleukin-6 (IL-6) and tumour necrosis factor alpha (TNFa) likely to be important.

In severe COPD, TNFa is present in sputum in high quantities [15]. TNFa activates neutrophils, macrophages and epithelial cells, causes MMP release from macrophages and inhibits skeletal muscle protein expression, thereby contributing to the loss of body mass seen in COPD (hence its previous name - cachexin). MMPs may also play a key role in the development of the characteristic COPD inflammatory process in a healthy smoker.

4 Conclusion

In COPD patients due to SM exposure inflammatory markers (highly sensitive CRP, interleukin 6) are elevated and these markers have direct association with the severity of disease. The finding which recommends the role of systemic inflammation in the pathogenesis of COPD due to SM intoxication like the COPD due to other causes.

The studies discussed in the present review clearly support the concept that chronic obstructive pulmonary disease can no longer be considered a disease affecting the lungs alone. The available evidence indicates that: 1) chronic obstructive pulmonary disease has an important systemic component; 2) clinical assessment of chronic obstructive pulmonary disease ought to take into consideration the systemic components of the disease; and the treatment of these extrapulmonary
effects appears to be important in the clinical management of the disease. A greater understanding of the processes involved will allow treatment to be guided with greater precision, rather than relying on bronchodilating agents from which many patients derive minimal benefit. High level of serum CRP is associated with an increased risk of mortality in COPD patients.

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