

REVIEW ARTICLE

Molecular and cellular mechanism of lung injuries due to exposure to sulfur mustard: a review

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Abstract

Sulfur mustard (SM), a potent chemical weapon agent, was used by Iraqi forces against Iranian in the Iraq–Iran war (1981–1989). Chronic obstructive pulmonary disease (COPD) is a late toxic pulmonary consequence after SM exposure. The COPD observed in these patients is unique (described as Mustard Lung) and to some extent different from COPD resulted from other well-known causes. Several mechanisms have been hypothesized to contribute to the pathogenesis of COPD including oxidative stress, disruption of the balance between apoptosis and replenishment, proteinase-antiproteinase imbalance and inflammation. However, it is not obvious which of these pathways are relevant to the pathogenesis of mustard lung. In this paper, we reviewed studies addressing the pathogenicity of mustard lung, and reduced some recent ambiguities in this field. There is ample evidence in favor of crucial role of both oxidative stress and apoptosis as two known mechanisms that are more involved in pathogenesis of mustard lung comparing to COPD. However, according to available evidences there are no such considerable data supporting neither proteolytic activity nor inflammation mechanism as the main underlying pathogenesis in Mustard Lung.

Keywords: COPD, bronchiolitis obliterans, mustard lung, apoptosis, sulfur mustard, oxidative stress

Introduction

Sulfur mustard ($C_4H_8Cl_2S$) (2,2'-dichlorethyl sulfide; HD; SM), a potent alkylating agent, was used as a chemical weapon by Iraqi forces against Iranian in the Iraq–Iran war. There are about 34,000 survivors who were exposed to SM during Iran–Iraq war (1981–1989). These victims are still suffering from late toxic effects of this warfare, including ophthalmic, cutaneous and respiratory sequels (Khateri et al., 2003). Among them, respiratory disorders are the most lethal and disabling consequences (Aghanouri et al., 2004). Various surveys have been indicated that bronchiolitis obliterans (BO) is the main late respiratory pathology among them (Ghanei et al., 2004; Ghanei & Harandi, 2007), but the BO observed in such patients is somehow different from BO resulted from other causes e.g. post-lung transplant BO. Because of specific characteristics of respiratory disorder in these patients, it was described as “Mustard Lung.” Despite the significant respiratory symptoms such as dyspnea, a majority of victims have normal pulmonary function

tests (PFTs). Pathological studies, PFT and the normal value of diffusion capacity of lung for carbon monoxide transfer index, proved that these patients do not suffer from pulmonary fibrosis (PF) (Ghanei & Harandi, 2007). In addition, arterial blood gas samples of patients do not exhibit hypoxia or hypercapnia except in subjects with severe respiratory failure. Considering radiologic findings which show no evidence for emphysema (Ghanei et al., 2010), the presence of pulmonary emphysema seems to be ruled out (Mehrani et al., 2009). The underlying pathophysiology in such disorder remained unclear until recent years. Cellular and molecular investigations yielded significant evidence regarding the precise nature of this disorder.

Several mechanisms have been hypothesized for the pathogenesis of chronic obstructive pulmonary disease (COPD) but there is debate about mechanisms involved in patients with mustard lung disease (Demedts et al., 2006). Herein, we first tried to review systematically the existing formal structure for pathogenicity of

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conventional COPD in each section, and then compared each mechanism and discussed these with specific findings in mustard lung. We introduced related molecular evidence of mustard lung in this setting as well. All methods of study such as tissue sampling, broncho-alveolar lavage (BAL) fluid and serum and inhalation analysis were tracked for comprehensive reviewing of mechanism(s) underlying mustard lung. Providing further cellular and molecular evidence of mustard lung will lead to further unraveling. This review has outlined recent cellular and immunological approaches that have been addressed in mustard lung.

Involved mechanisms

Inflammation

In COPD

Inflammatory mediators especially interleukin-8 (IL-8) and IL-6 play a primary role in various chronic pulmonary diseases such as COPD and asthma, and they are also suggested as biomarkers for diagnosis and disease activity (Car et al., 1994; Nocker et al., 1996; Danilko et al., 2007; Lee et al., 2008). Increased serum and BAL fluid levels of IL-6 have been shown during exacerbations of COPD (Danilko et al., 2007; Lee et al., 2008). Analysis of the cell profile in alveoli and small airways shows an increase in all cell types implicated in COPD, including macrophages, T-lymphocytes, B-lymphocytes and neutrophils (Retamales et al., 2001). In addition, increased leukotriene B₄, IL-8 and related CXC chemokines in COPD airways are chemotactic signals that play a potential role in neutrophil recruitment in COPD (Tanino et al., 2002; Traves et al., 2002). Recently, C reactive protein (CRP) is known as a predictor of disease severity and mortality. It is also a biomarker of systemic inflammation in some of the pulmonary diseases such as COPD (Higashimoto et al., 2009; Thorleifsson et al., 2009). Higher serum titers of rheumatoid factor (RF) were shown to be present in patients with connective tissue disorders and pulmonary involvement and it was associated with severity of pulmonary dysfunction (Coffey et al., 1989; Sakaida, 1995).

In mustard lung

Some studies on patients with mustard lung have been performed to evaluate possible role of inflammation in them. In a cross-sectional study, serum high-sensitivity CRP (hs-CRP) of 50 consecutive SM patients with stable respiratory disease was compared with 30 healthy subjects. It was found that the serum hs-CRP level was increased in SM patients and may have a direct correlation with disease severity (Attaran et al., 2009).

Emad and colleagues (2007b) reported significant differences in cytokine (IL-8, IL-1 β , IL-6, tumor necrosis factor α (TNF- α) and IL-12) levels of BAL fluid between patients with bronchiectasis and healthy control subjects. They also reported that cellular constituents of BAL fluid in SM-exposed patients were very similar to patients with idiopathic PF, and this finding may indicate the

presence of an ongoing active alveolitis in PF (Emad & Rezaian, 1999; Emad & Emad, 2007c). The findings of the studies performed by Emad and colleagues (2007a) concluded that the development of fibrosis is associated in SM victims. Similarly, they found a significant correlation between their findings and the severity of fibrosis (Emad & Emad, 2007d).

First, it is very important to note that lung fibrosis was previously the most conflicting issue in mustard gas (MG)-exposed patients. In the beginning, some studies addressed the probable association of MG exposure with PF. However, various precise complementary studies with imaging, pulmonary function and indisputable histopathological documents ruled out lung fibrosis in this setting (Ghanei & Harandi, 2007). Furthermore, it is possible that the design and discussion in their studies could have been biased by their strong positive attitude toward fibrosis. Second, although we agree with the presence of inflammation especially due to oxidative stress at the beginning and in the acute phase after exposure to SM, it is not a main finding as an ongoing process after years.

In contrast, recent well-designed investigations are incompatible with prominent inflammatory pathogenesis in mustard lung. Pourfarzam and colleagues (2009) evaluated the association of the serum levels of IL-8, IL-6, CRP and RF with long-term pulmonary involvement. Surprisingly, they found that the serum levels of IL-8 and IL-6 were significantly decreased in the SM-exposed participants compared with the control group. There were no significant associations between the serum levels of IL-8 and pulmonary symptoms (chronic cough, sputum, hemoptysis and dyspnea), pulmonary signs (rale and wheeze) as well as spirometry parameters. However, IL-6 was associated with wheezing and CRP was associated with both wheezing and rale in the SM-exposed group. It was concluded that serum levels of these inflammatory mediators probably do not play a major role in pathogenesis and persistence of pulmonary complications and could not reflect the degree of severity of pulmonary involvement following SM exposure (Pourfarzam et al., 2009).

It should be considered that although the pathologic nature of mustard lung is chronic bronchiolitis, the process is different from BO resulted from lung transplantation (Ghanei et al., 2008). Also, despite the similarity between SM-induced lung disease process and COPD i.e. fixed airway obstruction, probably ongoing systemic inflammation could not be justified in mustard-injured cases by the low levels of these cytokines when compared with the control group. The results would be very important in treatment of SM-exposed patients with COPD and also conventional COPD due to different etiologies without presence of active inflammation. This point is important when anti-inflammatory agents including corticosteroids are assumed to play a major role in treatment of COPD. Undesirable effect of corticosteroids in airway reversibility in more than 50% of mustard-injured cases (Ghanei et al., 2005) could be explained by the

absence of a persuasive active inflammation process in these patients. These results showed that there was no sign of active ongoing systemic inflammatory process in mustard-injured cases. This finding is in favor of the slow progression of disease in this population (Ghanei & Harandi, 2007).

Also there are conclusive evidences provided by histopathological studies on lung specimens of patients. A collaborative multicentric study revealed the histopathologic spectrum of changes in a large number of surgical lung biopsies from patients exposed to SM. The process was sometimes accompanied by only a mild-to-moderate lymphocytic infiltration (Ghanei et al., 2008). It was compatible with former pathological study (Beheshti et al., 2006). Thereby, it rejects the hypothesis that inflammation is the principal mechanism of injury in mustard lung. The long-held belief that all COPDs have inflammatory process may be questionable and we suppose that in a significant proportion of non-smokers with COPD like mustard lung, the role of inflammation is less important than current expectations. By considering this fact, it is more important that an estimated 25–45% of patients with COPD have never smoked, and the burden of non-smoking COPD is therefore much higher than previously assumed (Salvi & Barnes, 2009).

Increased proteolytic activity

In COPD

There is a disruption of the balance between proteolytic and antiproteolytic molecules in the lungs of patients with COPD that yields an increased proteolytic activity (Demedts et al., 2005a). It causes destruction of healthy lung parenchyma, which leads to the development of emphysema. Increased proteolytic activity may be a consequence of inflammation (release of proteolytic enzymes by inflammatory cells such as macrophages and neutrophils) or may be arisen from some genetic factors (e.g. α 1-antitrypsin deficiency) (Demedts et al., 2006).

In mustard lung

There is no evidence for emphysema in mustard lung resulted from different pathological (Ghanei et al., 2008) and radiological (Ghanei et al., 2004) studies carried out on this group of patients. Emphysema has not been a feature of SM lung complications in non-smokers and likely represents the effect of smoking or biomass smoke exposure (Ghanei & Harandi, 2007). In such circumstances, the roles of proteolytic and antiproteolytic molecules have also been waned.

Oxidative stress

In COPD

There is increasing evidence that oxidative stress is an important feature in COPD. Numerous studies have documented increased expression of markers of oxidative stress in the lungs of patients with COPD, compared with healthy subjects including 4-hydroxy-2-nonenal, a highly reactive lipid peroxidation end product (Rahman

et al., 2002), H_2O_2 and isoprostane (Dekhuijzen et al., 1996; Nowak et al., 1999; Biernacki et al., 2003). There are many actions of oxidative stress that can potentially play a role in the pathogenic mechanisms in COPD (MacNee & Tuder, 2009). It occurs when reactive oxygen species (ROS) are produced in excess of the antioxidant protective mechanisms and result in harmful effects, including damage to lipids, proteins and deoxyribonucleic acid (DNA) (Repine et al., 1997; Henricks & Nijkamp, 2001; MacNee, 2001), and cell dysfunction or cell death and can directly damage components of the lung matrix (e.g. elastin and collagen) and can also interfere with elastin synthesis and repair (MacNee, 2006).

Oxidative stress influences the proteinase-antiproteinase imbalance by the inactivation of antiproteases (such as α 1-antitrypsin or secretory leukoprotease inhibitor) (Henricks & Nijkamp, 2001) or activation of metalloproteases by oxidants (Shapiro, 2002, 2003). Moreover, oxidants have a major role in inflammatory lung injury by inducing the transcription of proinflammatory genes (Demedts et al., 2006).

Oxidative stress leads to the oxidation of arachidonic acid and the formation of a new series of prostanoid mediators called isoprostanes, which may exert significant functional effects (Morrow, 2000), including bronchoconstriction and plasma exudation (Kawikova et al., 1996; Okazawa et al., 1997; Janssen, 2001).

Oxidative stress may also induce apoptosis in endothelial and epithelial cells. The administration of a compound with antioxidant activity prevented the development of alveolar cell apoptosis and airspace enlargement, suggesting a positive feedback interaction between oxidative stress and apoptosis (Demedts et al., 2006).

Interestingly, recent studies suggest that there may be a link between oxidative stress and the poor response to corticosteroids in COPD. Oxidative stress impairs binding of glucocorticoid receptors to DNA and the translocation of these receptors from the cytoplasm to the nucleus (Hutchison et al., 1991; Okamoto et al., 1999).

Accordingly, N-acetylcysteine (NAC) is a potent antioxidant agent that acts as a prodrug for cysteine and glutathione (GSH). This mechanism of action has been proposed as the basis for its use in bronchopulmonary disease (Grandjean et al., 2000). Previous studies have shown that NAC could be effective in the treatment and control of clinical manifestations in patients with COPD by its antioxidative properties (Stey et al., 2000; Dekhuijzen, 2004; van Overveld et al., 2005), and could also reduce bronchial infection (Riise et al., 1994) and exacerbation (Pela et al., 1999) in these patients. In addition to its efficacy in COPD (Decramer et al., 2005), it can also improve fibrosing alveolitis (Behr et al., 1997) and idiopathic PF (Demedts et al., 2005b) by its antioxidant properties.

In mustard lung

Also, SM toxicity and pathogenesis seem to be mediated by generation of ROS (Naghii, 2002; Shohrati

et al., 2010). According to this hypothesis, GSH and malondialdehyde (MDA) levels were measured in SM-exposed patients comparing to non-exposed patients. In this study, decreased serum level of GSH and increased level of MDA made it clear that there are important alterations of oxidative-antioxidative system in patients suffering from SM-induced lung injuries. Patients with moderate-to-severe SM-induced lung injuries had a tendency to show decreased level of GSH and increased level of MDA rather than those with mild injuries (Dekhuijzen, 2006). GSH is an important antioxidant in the lung with a protective effect against both internal and external toxic agents (Jörres et al., 2000). GSH level is associated with the resistance of cells to alkylating agents. GSH depletion is not limited to SM intoxication, and repeated exposures to other toxicants like ozone (Bhisey et al., 1999) and tobacco (for workers of tobacco factories) (Fidan et al., 2005) can also decrease the GSH level. MDA, which arises from the breakdown of lipid peroxyl radicals, is one of the indicators of oxidative stress. MDA also can cause further oxidative injury by oxidizing protein molecules. Thus, it is both indicator and effector of oxidative stress (Ucar et al., 2007). It seems that increased MDA levels depict increased lipid peroxidation which may be due to excessive production of free radicals after exposed to SM (Anderson et al., 2000).

Accordingly, antioxidative effect of NAC was assessed in SM-exposed subjects. It has been shown that NAC reduces the number of neutrophils in lung injuries in mice exposed to SM (Han et al., 2004).

In a double-blind clinical trial study on 144 SM-induced BO patients with normal PFT, dyspnea, wake-up dyspnea and cough improved after 4 months of NAC administration compared with the placebo group. NAC reduced sputum from 76.9% of cases before the trial to 9.6% of cases after the trial. Spirometric components were significantly improved in NAC group compared with the placebo group (Shohrati et al., 2008).

Several studies have suggested an antioxidant effect of apolipoprotein (Apo) A1 and S100 calcium-binding proteins (Gabay & Kushner, 1999; Navab et al., 2004). Elevated levels of such proteins confirm the theory of considering the oxidative-antioxidative imbalance in the pathophysiology of SM-induced pulmonary lesion. By studying BAL fluid of SM-exposed patients, Mehrani and colleagues (2009) tried to identify different expressed proteomic proteins patterns in contrast to healthy control subjects. Apo A1 was detected in all MS-exposed patients' BAL fluid but not in any of the healthy control subjects. A significant increase in Apo A1 and haptoglobin isoforms was observed. The increase in these proteins was associated with the severity of pulmonary dysfunction. Furthermore, S100 calcium-binding protein A8 was only detected in BAL fluid of the moderate and severe groups. It seems that its expression might be induced in lung tissues in which the mild group with low damage also shows increasing Apo A1 expression. In contrast, S100

calcium-binding protein was dominantly expressed in the moderate and severe groups but not in the mild group.

Apoptosis

In COPD

Recent data from both animal models and studies on human subjects support an important role of apoptosis as fourth cardinal mechanism that might be involved in the pathogenesis of COPD. Two major pathways have been illustrated that could trigger apoptosis, namely within the cell. The extrinsic pathway is activated by ligand-activated death receptors such as Fas ligand- (FasL) Fas (Thorburn, 2004). The binding of Fas-FasL activates caspases, cysteine proteases that recognize aspartate at their substrate cleavage site, and induced apoptosis (Kumar, 1999). Takabatake et al. (2000) described that the serum levels of soluble Fas ligand (sFas-L) was not increased in patients with COPD. Others described a significant increase in sFas in plasma from patients with severe COPD compared with patients with mild or moderate COPD, whereas sFas-L was within normal limits in all groups (Yasuda et al., 1998). Caspases are protease enzymes that play important role in the apoptosis process. Both extrinsic and intrinsic apoptotic pathways activate caspase-3 which is the executioner caspase responsible for the end effects in the apoptosis process (Budihardjo et al., 1999; Porter & Jänicke, 1999; Ray et al., 2010).

However, apoptosis is not an isolated process in the development of COPD and it interacts with all pathways like oxidative stress, adding to the complexity of the disease (Demedts et al., 2006). Apoptosis is believed to be a major mechanism for the clearance of neutrophils from sites of inflammation. It was demonstrated that apoptosis predominates in the areas of oxidative stress in lung. Experimental blockade of apoptosis markedly reduced the expression of markers of oxidative stress (Tuder et al., 2003). It should be considered that there is a positive feedback interaction between oxidative stress and apoptosis. In other words, there is a relative suppression of apoptotic cell clearance under oxidant stress in acute lung injury. Consequently, NAC has been used as a potent antioxidant agent and promotes apoptotic cell clearance through downregulation of the RhoA/Rho kinase pathway, resulting in resolution of lung inflammation. Previous *in vitro* data indicate that endogenous and exogenous oxidants can inhibit uptake of apoptotic cells by macrophages and a variety of antioxidants normalized apoptotic cell uptake (Anderson et al., 2002; McPhillips et al., 2007). Moon et al. (2010) found that there is a relative suppression of apoptotic cell clearance through oxidant-dependent activation of RhoA/Rho kinase in acute lung injury in mice. They also demonstrated how an antioxidant such as NAC enhances apoptotic cell clearance through the downregulation of RhoA in alveolar macrophages. It results in a concomitant decrease of proinflammatory mediators and increase in transforming growth factor β -1 (TGF- β 1) production as well

as reduction of inflammatory cell accumulation (Moon et al., 2010).

On the other hand, efferocytosis is engulfment of apoptotic cells by phagocytes followed by cell replacement to maintain homeostasis. Without efferocytosis, the apoptotic neutrophils undergo secondary necrosis resulting in induction of the proinflammatory cascade (Haslett, 1999). Efferocytosis is suppressed by oxidants and consequently antioxidants exposure would increase the ability of efferocytosis. The TGF- β improves the efficiency of efferocytosis in the lung (Naghii, 2002; Shohrati et al., 2010). Several lung diseases, including asthma and COPD (Vandivier et al., 2002; Hodge et al., 2003; Demedts et al., 2006) are related to impaired efferocytosis. Bergman et al. (1998) detected increased levels of TGF- β 1 transcripts in BAL cells of lung transplant recipients affected with BO. Overproduction of TGF- β in BO patients has also been shown by El-Gamel and colleagues (1999). But they did not distinguish the exact upregulated isoform of this factor (El-Gamel et al., 1999).

In mustard lung

In vitro and *in vivo* studies showed that SM induces time- and dose-dependent apoptosis (physiological cell death) and necrosis (pathological cell death) in cells (Kehe et al., 2000; Steinritz et al., 2007; Heinrich et al., 2009). Both intrinsic and extrinsic pathways seem to be involved in SM-induced apoptosis (Kehe et al., 2009; Ray et al., 2010).

Expression of different types of TGF- β transcripts was examined in chemically injured patients and was compared with healthy volunteers. The higher level of TGF- β 1 protein in BAL fluid of a group of veterans exposed to chemical gas was detected using ELISA technique (Aghanouri et al., 2004). In view of TGF- β properties in a recent unpublished study, it was hypothesized that TGF- β may be responsible for airway remodeling, homeostasis and slow progression of respiratory disease in chemically injured patients. These results indicated that levels of TGF- β 1 and TGF- β 3 mRNA were significantly higher in chemical gas-injured patients rather than non-injured group. As a consequence, it was suggested that TGF- β 1 and TGF- β 3, but not TGF- β 2, may improve the efferocytosis and play an inhibitory role in airway remodeling and lung homeostasis in chemically injured group. These properties of TGF- β are consistent with long time survival of chemically injured patients suffering from BO. On the other hand, the induction of apoptosis along with defective clearance of apoptosis (defective efferocytosis) has been shown to be associated with various lung disorders including COPD (Hodge et al., 2003; Demedts et al., 2006). Some studies have shown that macrophage ingestion of apoptotic cells causes an increased release of TGF- β (Fadok et al., 1998; McDonald et al., 1999) which results in induction of effective efferocytosis, suppression of inflammatory and immunogenic response, proliferation of epithelial and endothelial cells, and the maintenance of normal lung structure (Freire-de-Lima

et al., 2006). These findings are compatible with cystic fibrosis and COPD diseases in which the TGF- β protein level is lower than normal which, in turn, causes ineffective clearance of apoptotic cells and leads to disease state (Henson et al., 2006; Vandivier et al., 2006).

Moreover, as mentioned earlier, intracellular levels of GSH have been shown to affect the sensitivity of cells to cell death-inducing stimuli, as well as the mode of cell death. In fact, induction of cell death markedly accelerates due to GSH depletion (Vahrmeijer et al., 1999); the circumstance that has been shown in SM-exposed patients.

Rosenthal et al. have shown that SM induces type I apoptotic cell death through the Fas/FasL-system (Rosenthal et al., 2003). It has been shown that caspase activity is involved in the cell death pathway that is induced following exposure to mild concentrations of SM (Rosenthal et al., 1998, 2003; Steinritz et al., 2007; Ray et al., 2008; Mol et al., 2009). Ray et al. (2008) reported that acute exposure to various mustard concentrations would induce apoptotic pathway in normal human bronchial epithelial cells and small airway epithelial cells via caspase-mediated pathway *in vitro*. More recently, they suggest a death receptor pathway of apoptosis that utilizes a feedback amplification mechanism involving an activated death receptor complex that leads to the activation of caspase-9 via a caspase-3 pathway (Rosenthal et al., 1998). Sourdeval et al. (2006) reported that mustard-induced apoptosis involves a post-mitochondrial caspase-dependent pathway in the detached cells. It is also demonstrated that caspase inhibition could prevent MMP induction. Kehe and colleagues (2008) showed that acute SM exposure induces apoptosis by caspase-dependent pathway.

A second type of regulatory proteins that might be affected in SM-exposed patients could be FLICE inhibitory protein (FLIP). FLIP is able to block early events in Fas and TNF-receptor-like apoptosis-inducing ligand/TNF family death receptor signaling by precluding caspase-8 recruitment to the DISC. FLIP is a caspase-8-like protein that interferes with caspase-8 binding to the DISC, thus preventing caspase-8 oligomerization and autoactivation (Krueger et al., 2001; Kim et al., 2002). Finally, SM exposure might activate the inhibitor of apoptosis proteins (IAPs), which constitutes a family of evolutionarily conserved apoptotic suppressors. Members of the IAP-family proteins, such as X-linked inhibitor of apoptosis protein (XIAP), have been shown to bind to and inhibit activated caspase-3, -7 and -9 (Deveraux et al., 1998). Phosphatidylserine is normally exposed on inner side of plasma membranes, and it becomes exposed on outer membrane surface of the cells undergoing apoptosis. Therefore, annexin V-FITC is able to detect the surface changes in membrane surface that occurs early during apoptosis (Fadok et al., 1992).

In vivo study with rodent pulmonary tissue exposed to SM showed increased gene expression of apoptosis-related genes (Dillman et al., 2005). However, little is

known about the signal transduction pathways activated by long-term effects of SM. In a case-control study by Pirzad and colleagues (2010), Fas and FasL levels, caspase-3 activity and percent of apoptotic cells were measured in BAL fluid of patients 20 years after exposure to SM and compared with the control group. There was significant increase in BAL fluid cells Fas and FasL levels in mild and moderate-to-severe patients compared with the controls. FEV1/FVC ratio which is more sensitive for airway obstruction was significantly higher in patients with mild disorder compared with moderate-to-severe respiratory disease. It indicates that the patients with moderate-to-severe symptoms are more prone to airway obstruction. In addition, there was no difference in BAL fluid cells caspase-3 activity among patients and control groups. As caspase-3 is considered to be one of the main effector caspases in apoptotic cell death, it could be concluded that apoptosis was impaired in BAL fluid cells of patient population. Further analysis of BAL fluid cells with annexin V-FITC versus propidium iodide confirmed that the majority of cells became necrotic and only a small portion became apoptosis.

Two mechanisms have been proposed for the elevation of Ca^{2+} levels in relation to apoptosis. The first mechanism involves protein kinases signaling pathways that leads to the activation of phospholipase C and the generation of inositol triphosphate, which acts on Ca^{2+} channels to release Ca^{2+} from intracellular stores. The second mechanism involves oxidative stress in which ROS generated by toxicant exposure react with Ca^{2+} transport channels in the endoplasmic reticulum, mitochondria and cell membrane. According to Mehrani and colleagues' proteomic study (2009), there was a significant increase in vitamin D binding protein isoforms, haptoglobin isoforms and fibrinogen especially in SM-exposed patients with moderate and severe lung diseases. In addition, a significant decrease was noted in calcyphosine, surfactant protein A and transthyretin in these patients. It was discussed that calcium-binding proteins are the main proteins involved in the process of SM pathogenicity. S100 protein isoforms are implicated in the immune response, differentiation, cytoskeleton dynamics, enzyme activity, Ca^{2+} homeostasis and growth (Emberley et al., 2004). Moreover, evidence has recently revealed that this family of proteins has a broad spectrum of activity in regulating apoptosis and tissue remodeling (Yui et al., 2003).

Conclusion

Exact mechanisms leading to mustard lung have not been cleared so far. It is not obvious exactly which pathway and how it could cause pathogenicity. Although inflammation is known as one of the main pathogenesises involved in COPD, there is no conclusive evidence that inflammation make significant contributions to the pathogenesis of mustard lung. Nevertheless, based upon results from a growing number of studies, we believe that

oxidative stress and apoptosis are two more acceptable mechanisms of pathogenesis in this setting. We hypothesize that there is somehow an adequate longtime balance between inflammatory and anti-inflammatory processes and also between oxidant and antioxidant processes in mustard lung. Moreover, with a positive feedback interaction between apoptosis and oxidative stress, apoptosis is predominated in areas of oxidative stress (Shohrati et al., 2008). However, there is a proliferation in bronchioles because the efferocytosis process could not reach to the end. It is the reason that up to 80% of patients have been remained with mild pulmonary function impairment and only 20% were categorized in the moderate-to-severe group (Ghanei & Harandi, 2007). The understanding of the cellular and molecular mechanisms involved in mustard lung is in the beginning compared to comprehensive knowledge of conventional COPD or asthma. Although both diseases involve the respiratory tract, the pattern of inflammation, the results of the inflammatory process and the therapeutic response are markedly different.

Future studies

Supplementary studies are necessary to understand more about apoptosis and its different pathways in this setting. Further attempts to determine oxidative stress and related genetic characteristics are fully appreciated. Final mission is to obtain novel diagnostic methods and effective remedies for such patients that would also be applied for conventional COPD.

Declaration of interest

There is no actual or potential conflict of interest relevant to this article. Authors report no disclosures.

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