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REVIEW ARTICLE

A clinicopathological approach to sulfur mustard-induced organ complications: a major review

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Abstract

Context: Sulfur mustard (SM), with an old manufacturing history still remains as potential threat due to easy production and extensive effects.
Objectives: Increasing studies on SM indicates the interest of researchers to this subject. Almost all human body organs are at risk for complications of SM. This study offers organ-by-organ information on the effects of SM in animals and humans.
Methods: The data sources were literature reviews since 1919 as well as our studies during the Iraq–Iran war. The search items were SM and its all other nomenclatures in relation to, *in vivo*, *in vitro*, humans, animals, eye, ocular, ophthalmic, lungs, pulmonary, skin, cutaneous, organs and systemic. Amongst more than 1890 SM-related articles, 257 more relevant clinicopathologic papers were selected for this review.
Results: SM induces a vast range of damages in nearly all organs. Acute SM intoxication warrants immediate approach. Among chronic lesions, delayed keratitis and blindness, bronchiolitis obliterans and respiratory distress, skin pruritus, dryness and cancers are the most commonly observed clinical sequelae.
Conclusion: Ocular involvements in a number of patients progress toward a severe, rapid onset form of keratitis. Progressive deterioration of respiratory tract leads to "mustard lung". Skin problems continue as chronic frustrating pruritus on old scars with susceptibility to skin cancers. Due to the multiple acute and chronic morbidities created by SM exposure, uses of multiple drugs by several routes of administrations are warranted.

Keywords

Clinicopathologic, complication, cutaneous, ocular, organs, pulmonary, sulfur mustard

History

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Introduction

Sulfur mustard (2,2-dichlorodiethyl sulfide; SM) has been used as a chemical warfare agent since the early twentieth century; in the past decade, it has reemerged as a major threat around the world. SM is an agent that is easily produced even in underdeveloped countries and for which there is no effective therapy. This agent is a potential threat not only on the battlefield but also to civilian populations[1]. SM was extensively used against Iranian soldiers and civilians between 1983 and 1988, resulting in over 100 000 chemical casualties[2].

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About 45 000 Iranians are still suffering from late respiratory complications due to SM exposure[3]. In one air attack to Sardasht city of Iran alone, about 8000 out of 12 000 residents of the city were contaminated. At least 4500 exposed population needed treatment[4]. Overall, the organs that were mainly affected in a very large series of patients include lesions of the lungs (42.5%), eyes (39.3%) and skin (24.5%)[5]. In 600 civilian exposed patients, ocular, cutaneous and respiratory manifestations were present in 96.2, 83.8, 80.7% of the cases, respectively, with a hospitalization rate of 64.7%[6]. Although late eyes, respiratory and skin complications were mainly due to direct toxic effects, the neuromuscular, hematological and immunological complications were probably the result of systemic toxicity[7]. A dramatic presentation of SM attack had been discussed in Munich experience: immediately after the attack, the victims reported to feel an unpleasant smell of

garlic, addled eggs or oil-roasted vegetables. Following an asymptomatic interval of few hours, acute subepidermal skin blisters, respiratory tract damage and ocular symptoms started. Eye itching and skin blisters occurred 2 h after SM exposure, whereas some patients had nausea, dizziness and hoarseness. About 4 h later, most patients started vomiting. Eye symptoms worsened and most patients suffered from temporary blindness due to blepharospasm and lid edema. Red eye and photophobia were the major eye symptoms. Pulmonary symptoms, including productive cough were persistent. In the clinical examinations, bronchoscopy revealed massive inflammation of the trachea with signs of necrosis. Bone marrow depression followed later[8]. The eyes are very sensitive to SM products, which may be due to exposed corneal and conjunctival surfaces and high absorbance and metabolic turnover of these tissues[9]. In acute phase, the eyes were the first affected organ with a sensation of grittiness, lacrimation, photophobia, blepharospasm and corneal ulceration. In chronic phase, delayed keratitis in severely intoxicated patients had been diagnosed with corneal vascularization, thinning and epithelial defect many years after initial exposure. Respiratory symptoms such as rhinorhea, laryngitis, tracheobronchitis and dyspnea were also started. Late respiratory complications include chronic obstructive pulmonary disease (COPD), bronchiectasis, asthma, large airway narrowing and pulmonary fibrosis[10]. SM inhalation significantly increased tidal volume, peak inspiratory and expiratory flow, end expiratory pause and enhanced pause gradually by the time of exposure. These changes were minimal at the first 5–6 h and peaked at the 23–24 h after exposure. Later changes included airways obstruction with loose cellular debris, damaged cells and exudates[11]. Both the severity and frequency of bronchiectatic lesions increased during long-term follow-up. An association between human lung cancer and SM has been outlined since World War I[12]. Acute skin lesions based on the severity of exposure varied from erythema, bullous lesions and even necrotization[10]. Long-term skin lesions include xerosis and pruritus, pigmentation disorders, scars, and cherry angiomas. Although SM is a well-known carcinogenic agent, skin cancers have been reported infrequently[13]. Cutaneous complications revealed no significant correlation with either ocular or respiratory complications[9], but significant positive correlation has been reported between the severity of ocular and respiratory complications[9,14].

Toxic effects of SM depend on its dose and duration of exposure. The amount of material required to produce a specified effect in 50% of an animal population (ED50) is 50 and 75 μM for dividing and confluency of cells, respectively[15]. Severe mustard poisoning in humans is usually observed at mustard doses of 1000 mg/min/m^3 in air, causing damage to hematopoietic tissues and progressive leucopenia. As SM threats against military and civilian populations are possible forever, physicians should be aware of its severe effects and well-informed how to treat the victims[16]. Given the toxic effects of SM on different tissues, this study provides evidence about SM lesions on different organs in animals and humans, for physicians and researchers.

Literature reviews such as Pub Med, Medline and ISI database provided the data sources. In relation to SM and related synonyms, more than 1890 articles in English have been found since 1919 as well as our studies during the Iraq–Iran war in Persian language. Among them, *in vivo* and *in vitro* human (662 studies), animals (514), skin and cutaneous (428), lungs and pulmonary (249), eye, ocular and ophthalmic (101) and systemic (50) studies were in order of frequencies. Among these papers, 257 full-text articles which were more relevant to clinical-pathologic aspects of SM were selected for this review.

Toxicology

Chemical and physical properties of SM

SM is an alkylating, blistering or vesicating agent. Other related or historical names of SM include mustard gas (MG), *bis*, yperite, H (contains about 20–30% impurities, mostly sulfur), HD (distilled mustard that is partly pure), agent T (a closely related vesicant with a lower-freezing point) and HT (a mixture of 60% HD and 40% agent T)[17]. SM is an oily, clear to pale liquid which in impure form shows a yellowish to black discoloration. Chemical formulation of the most famous type of SM (HD) molecule is $C_4H_8Cl_2S$, with a molecular weight of 159.08 Da. Other physical and chemical properties of SM includes: melting point of 13–14 °C, boiling point of 215–217 °C, density of 1.27 g/ml in liquid form at 20 °C and a vapor pressure of 0.11 mmHg in 20–25 °C[17].

Biochemical and cellular disturbances

Shortly after SM exposure, cell DNA alkylation has been identified as a major trigger of apoptosis, which includes mono-functional SM-DNA adducts as well as DNA cross-links. As a consequence, DNA replication is blocked, which leads to cell-cycle arrest and DNA single- and double-strand breaks. The SM-induced DNA damage results in poly-(ADP–ribose) polymerase (PARP) activation. High SM concentrations induce PARP over activation, thus depleting cellular nicotinamide adenine dinucleotide (NAD+) and ATP levels, which in turn results in necrotic cell death. SM-induced apoptosis has been linked both to the extrinsic cell surface death receptor (Fas) or intrinsic (mitochondrial) pathway. In addition, in acute phase SM upregulates many pro-inflammatory mediators including interleukin (IL)-1α, IL-1β, IL-6, IL-8, tumor necrosis factor-alpha (TNF-α) and nuclear factor kappa-B (NF-κB) cells activation[18]. Oxidative stress involves inducible nitric oxide synthase (iNOS) which also leads to peroxynitrite (ONOO−) production that in turn activates NF-κB and activator protein-1 (AP-1), and expression of pro-inflammatory genes lead to promoting of inflammation. In addition, ONOO− could directly damage all biomolecules including lipids, proteins and DNA within the cells. Individuals with moderate-to-severe SM-induced lung injuries showed a decreased serum level of glutathione (GSH) and an increased serum level of malondialdehyde (MDA) activities compared with the mild injuries 20 years after exposure, which represents their tendency to oxidative stress[19]. Superoxide dismutase, catalase and GSH peroxidase activities in bronchoalveolar lavage (BAL) fluid, plasma and erythrocytes are significantly higher in exposed patients. The increased glutathione-*S*-transferase activity in BAL fluid was associated with a depletion of GSH and an increase of MDA

levels[20]. Overall decrease in the activity of superoxide dismutase by SM exposure is probably mediated by direct inactivation of the extracellular type of this gene or enzyme itself, which may be due to Cys–Cys disulfide bonding cleavage in lung cells[21]. In chronic SM toxicity, significant reduction in serum albumin and paraoxonase-1 activity may lead to long-term accumulation of reactive oxygen metabolites which in turn subsequently lead to oxidative stress state. These changes might contribute to morbidity and occurrence of other complications, such as atherosclerosis and rapid or premature aging in these patients[22]. Using human mononuclear leukocytes, post-exposure cell death process induced by HD did not initiate before 4 h, but continued in a dose-dependent manner afterward. Antioxidants (niacinamide and 3-aminobenzamide, PARP inhibitors) are effective in preventing cell death partially if administered as early in the first 12 h[23].

Exposure confirmation

Animal and in vitro *studies*

Urinary excretion of thiodiglycol (TDG), a major HD metabolite appears to increase within the first 1–4 d following exposure, with detectable levels after 1 week. Relatively high urinary TDG levels may thus indicate agent exposure within the previous 96 h. Lower urinary levels may indicate either exposure to low levels of agent or exposure that occurred more than 4 d prior to collection of the sample[24]. Detection of the major adduct, N7-[2-[(hydroxyethyl)thio] ethyl] guanine (N7-HETE-Gua), formed after alkylation of DNA with SM is an immunochemical method for dosimetry of exposure to SM[25]. In an optical coherence tomography (OCT) technique, a flexible fiber optic OCT probe is introduced into the distal trachea to image airway epithelium and mucosa *in vivo*. This high-resolution imaging modality helps for detecting, assessing and monitoring the treatment of airway injury following SM exposure[26].

Human studies

A standard operating procedure has been developed for an immuno-slot blot assay of SM adducts to DNA in human blood and skin for use in a field laboratory. A minimum detectable level of exposure of human blood ($>$ or $=$ 50 nM) to SM is feasible with the assay. In the case of human skin, even 1 s exposure to saturated SM vapor (830 mg/m^3) could still be detected[27]. In the autopsy results of an Iranian soldier who died 7 d after SM exposure, the following concentrations of SM were found (mg SM/kg tissue wet weight): brain – 10.7, cerebrospinal fluid – 1.9, liver – 2.4, kidney – 5.6; spleen – 1.5, lung – 0.8, muscle – 3.9, fat – 15.1, skin – 8.4, skin with subcutaneous fatty tissue – 11.8, liquid from a skin blister – below detection limit, blood – 1.1 and urine – below detection limit[28].

Immunologic disturbances

Animal and *in vitro* studies

In early stages of contact, SM not as a chemotaxin but through a normal inflammatory function causes a cellular infiltrate consisting large amount of polymorphonuclear leukocytes (PMN) and exocytosis of PMN azurophilic and specific granules. PMN infiltration into the sites of SM lesions occurs as early as 30 min and peaks within several hours[29]. In skin lesions produced by the topical application of 1% SM in rabbits, endothelial intercellular adhesion molecule (ICAM) levels decreases by 50% in the first 2 d (peak lesions), but returns to normal within 3–6 d (healing process). Endothelial leukocyte adhesion molecule (ELAM) peaks within 1–2 d and remains high during the healing phase. Vascular cell adhesion molecule (VCAM) levels elevates on the day 6. ELAM plays a major role in acute inflammation, where ICAM and VCAM play major roles in chronic inflammation[30]. A time-related increase in overall matrix metalloproteinase (MMP)-9 mRNA and protein activity was observed in SM-treated mouse's ear skin within 7 d post exposure[31]. PMN and mononuclear cells (MN) are the major sources of the chemotaxins produced by the SM lesions. The culture fluids from early, peak and healing SM lesions in the skin of rabbits all showed high chemotactic activity for both PMN and MN and fibroblasts. The chemotaxins identified were the eicosanoid leukotriene (LT)-B4, the chemokine IL-8 and proteases producing the complement fragment C5a. The number of cells containing IL-1, IL-8, monocyte chemotactic protein (MCP)-1 and a granulocyte chemoattractant (GRO) mRNAs increased in SM lesions[32]. SM primarily decreased the functional activity of Th1 lymphocytes, immune reactions associated with these cells and interferon-gamma (IFN-γ) production compared with that of Th2 lymphocytes and IL-4 synthesis[33].

Human studies

Shortly after exposure, serum IgG levels initially decreased but gradually increased significantly. The initial decrease may be due to a possible leakage of IgG into the skin blisters or inflamed respiratory system, whereas the subsequent increase may be due to immune system reaction. Serum IgA alterations, however, not significant, were in parallel with IgG, but IgM did not show marked alterations during the first month after exposure[34]. In chronically exposed subjects, total counts for WBC, RBC, HCT, monocytes and CD3(+) T-lymphocytes, IgM, C3, absolute level of alpha-1, alpha-2 and beta-globulins were significantly elevated, but the percentage of $CD16^+56^+$ cells was significantly decreased[35]. In another study, the percentage of PMN lymphocytes was significantly elevated whereas the percentage of total $CD4^+$ cells were significantly diminished[36]. In severe intoxicated patients, the percentage of NK cells ($CD45^+/CD56^+$) was significantly decreased, whereas the activity of NK cells ($CD56^+/CD25^+$) was noticeably increased[37]. Ten years later, the percentage of $CD45^+$ was reported normal, whereas the percentage of T-helper and T-cytotoxic cells was significantly decreased. Also $CD4^+/CD25^+$ cells in the most severely affected patients were significantly increased[38]. Twenty years after exposure, the serum levels of soluble Fas ligand (sFasL) in an exposed group with pulmonary problems were significantly elevated and a positive correlation was reported between sFasL levels and pulmonary problems. There was also a significant negative correlation between sFasL and WBC count in the exposed group compared with controls[39].

In the same exposed group, the serum levels of IL-1α, TNF-α and IL-6, but not MMP-9, decreased compared with controls, whereas the serum titers of the C-reactive protein (CRP) and rheumatoid factor were significantly increased[40]. Also IL-1β and IL-1Ra were significantly lower than the controls and there were significant positive correlation between the concentrations of these cytokines[41]. Moreover, positive correlations were present between the serum levels of granulocyte-macrophage colony stimulating factor (GM-CSF) and the percentage of eosinophil, IL-1α, IL-1β, TNF-α and IL-6[42]. Also recently, the relationship between many systemic (serum) immunological parameters and ocular, pulmonary and skin involvement have been reviewed in this group of patients compared with controls[43–51]. It has been reported that tissues expression of transforming growth factor (TGF)-β1,2 and its receptors R1, R2 have been shown to have anti-inflammatory effects, which significantly decreases in chemical victims[52]. The serum levels of sL-selectin and sP-selectin were significantly reduced in the SM exposed patients, whereas sE-selectin was significantly increased. sL-selectin positively correlated with the percentage of PMN cells and negatively with the percentage of lymphocytes. The change in the pattern of selectins in the SM exposed may indicate suppressed acute inflammatory condition[53]. It was proposed that severe alteration in the immune system is the major cause of opportunistic infections, septicemia and death in patients who were severely exposed to SM[37].

Ophthalmologic complications

Animal and *in vitro* studies

The toxic ocular events following SM exposure are characterized by several stages: photophobia begins in a few hours after exposure; an acute injury phase characterized by inflammation of the anterior segment and corneal erosions develops within 48 h. Corneal nerve fiber degeneration appears during 3–7 d. The delayed phase begins following a clinically silent period and peaks within 1–2 months. Two types of corneal involvement exist: those exhibiting delayed ocular lesions (clinically impaired) and those exhibiting only minor injuries (clinically non-impaired). Clinically impaired corneas were characterized by chronic inflammation, increased MMP activity, poor innervations and limbal damage. The late injury was expressed by epithelial defects and corneal neovascularization (NV) and led to vision deficits and even blindness. Moreover, using impression cytology and histology, delayed lesions known as an ocular surface disorder were identified as the category of limbal epithelial stem cell deficiency (LSCD)[54]. In rabbit, 2.5-min exposure to SM developed MG keratopathy within 5 weeks. Recovery began within 2 weeks in few of the cases. Persistent edema and profound disorganization of the basement membrane zone developed at 8 weeks post exposure[55].

Human studies

Acute phase

The eyes are the first affected organ with grittiness sensation, lacrimation, photophobia, blepharospasm and finally corneal ulceration[10]. In this phase, the major eye symptoms include burning, itching, red eye and photophobia. Eye itching begins within 1–2 h after SM exposure. Ocular symptoms worsened and temporary blindness ensures in most patients due to blepharospasm and lid edema[8]. Early ocular symptoms and signs resemble an acute conjunctivitis[8,16]. The severity of clinical problems related to the dose, concentration and duration of the MG exposure[56]. Exposure to 12–70 mg/min/m^3 caused mild conjunctival injection without significant chemosis or any corneal involvement and any later squeals[57,58]. Exposure to 100–200 mg/min/m^3 caused moderate lesions recognized by severe conjunctivitis, blepharospasm and corneal epithelial shedding and even corneal ulcer and perforation[59,60]. The symptoms decreased after 48 h and corneal epithelium improved after a few days and all the symptoms resolved within 6 weeks[58,61] exposure to concentrations more than 200 mg/min/m^3 of SM caused systemic problems in addition to involvement of deep corneal layers. In these cases, ocular involvement includes eyelids ulcerations, severe conjunctival chemosis, limbal tissues necrosis, corneal vascularization and opacity, uveitis and even glaucoma and cataract formation[7,58,62,63].

Chronic phase

Common ocular symptoms and signs in chronic phase were itching (42.5%), burning (37.5%), photophobia (30%), tearing (27.5%), chronic conjunctivitis (17.5%), perilimbal hyperpigmentation (17.5%), limbal vascular tortuosity (15%), limbal ischemia (12.5%), corneal subepithelial opacity (15%) thinning (15%), diffuse corneal opacity (10%), NV (7.5%) and epithelial defects (5%)[9]. Photophobia and ocular surface discomfort are the most significant symptoms, whereas bulbar conjunctival abnormalities followed by limbal tissue changes are the most significant signs[64]. Varying degrees of LSCD was demonstrated in all patients with chronic or delayed-onset MG-induced keratopathy using impression cytology. Corneal clinical manifestations are more severe in nasal and temporal quadrants. There was no relation between impression cytology findings (positive versus negative for goblet cells) and corneal clinical grading[65]. In chronic phase, pathologic findings in conjunctiva include chronic and perilimbal ischemia, telangiectasis, vasculitis, subconjunctival hemorrhage, goblet cells loss, epithelial thinning or thickening, scars of substantia propria, lymphocytic infiltration and dilated lymphatic vessels. Pathologic findings of chronic phase in cornea include corneal epithelial and Bowman's layer destruction, keratocytes loss, conjunctivalization, vascularization, squamous metaplasia, thinning, ulceration, cellular infiltration, lipoid or amyloid deposition, endothelial cell loss, calcific band keratopathy and stromal scars[54,61,63]. Other ocular findings in chronic SM contamination include chronic blepharitis, meibomian gland dysfunction and dry eye[66]. Chronic blepharitis and decreased tear secretion are the two most important and influencing factors in the progression of ocular problems in SM injuries[67]. Also, unusual and potentially more dangerous conjunctival and ocular surface bacterial florae are isolated in these patients[68]. Many years after initial exposure, delayed keratitis may ensure in severely intoxicated patients with corneal vascularization, thinning and epithelial defect[10].

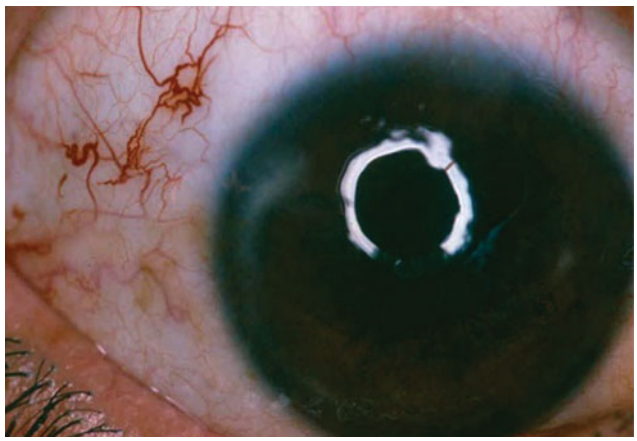

Figure 1. Mild SM-induced ocular injuries. Conjunctival vascular microaneurysm, telangiectasia, tortuosity, segmentation, dilatation and ischemia. (By courtesy of Janbazan Medical and Engineering Research Center.).

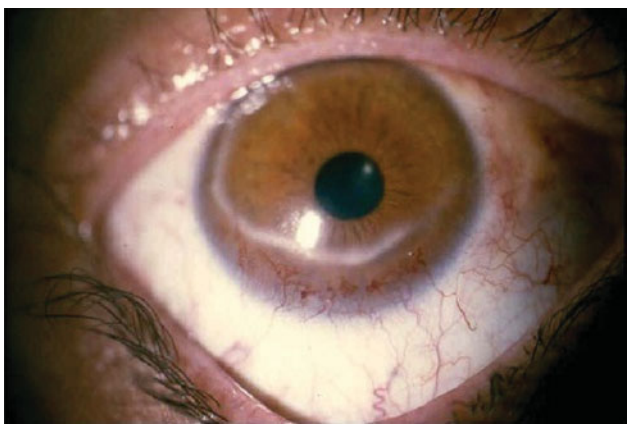

Figure 2. Moderate SM-induced ocular injuries. Mild ocular findings in addition to corneal epithelial, subepithelial and stromal opacities, and neovascularization and limbal ischemia. (By courtesy of Janbazan Medical and Engineering Research Center.).

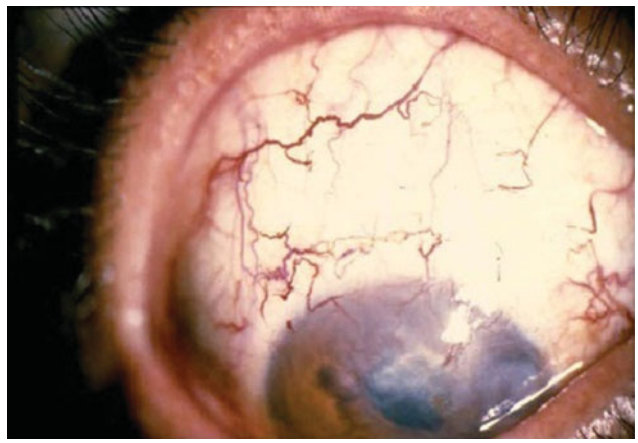

Figure 3. Severe SM-induced ocular injuries. Moderate ocular findings in addition to corneal melting, thinning and hyaline-like substance deposition and diffuse corneal opacity. (By courtesy of Janbazan Medical and Engineering Research Center.).

Corneal confocal scanning in a group consisting of 28 patients with chronic and delayed MG keratopathy revealed corneal thinning, significant keratocytes loss with pleomorphic changes, and thickening of midstromal nerve, stromal microdots and amyloid degeneration. Meanwhile, anterior and middle corneas were more involved than the posterior cornea[69]. Severity of the disease can be graded as follows: presence of symptoms of photophobia, foreign body sensation, burning, itching, lacrimation, redness and dryness. Mild: symptoms in addition to conjunctival vascular microaneurysm, telangiectasia, tortuosity, segmentation, dilatation and ischemia (Figure 1). Moderate: mild form in addition to corneal epithelial, subepithelial and stromal opacity, and NV and limbal ischemia (Figure 2). Severe: moderate form in addition to corneal melting, thinning, hyaline-like substance deposition or diffuse corneal opacity[67] (Figure 3).

Pulmonary complications

Animal and *in vitro* studies

Acute lung injury that occurs within 12–24 h after inhalation of 300 μM SM or within 8 h of 1000 μM SM is characterized by massive, localized hemorrhage and alveolar edema, which implies severe disruption of the vascular and distal airway barrier. A 50% reduction in lung barrier functionality occurs within this time[70]. Direct exposure of anesthetized large white pigs to gradually increasing HD vapor for 6 h caused progressively more hypoxemia with respiratory acidosis. Pathological findings include necrosis and erosion of the tracheal epithelium in moderate to high doses of SM. These findings are compatible with an acute lung injury that occurs at 3–6 h post-exposure[71]. *In vitro* studies showed that both upper airway and deep lung epithelial cells undergo SM-induced apoptotic cell death[72]. In exposed (head only) Guinea pigs to various concentrations of SM vapor, clinical symptoms at 3 h post exposure were erythematic and swelling nose with extensive mucous secretion (with or without bleeding) followed by breathing difficulties, rhonchi and dyspnea at 6 h post exposure. The symptoms peaked within 48 h and remained up to 8 d, followed by spontaneous apparent recovery of respiratory parameters and normal weight gain within 2 weeks. Abnormal epithelial growth and cellular infiltration into the lung as well as significant changes in protein, LDH and GSH levels in the BAL fluid at 4 weeks post exposure could cause recurrent lung injury similar to that reported for HD-exposed human casualties[73]. Exposure of rats' lungs to SM caused focal ulceration and detachment of the trachea and bronchial epithelia, thickening of alveolar septal walls, increased numbers of tissue inflammatory cells, autophagy and apoptosis in the tissue and increased BAL protein content as a marker of injury to the alveolar epithelial lining starting within a few hours to days. SM exposure results in increased expression of inflammatory markers of cyclooxygenase-2 (COX-2), TNF-α, iNOS and MMP-9 in alveolar regions, whereas MMP-9 is prominent in bronchial epithelium. In contrast, expression of the antioxidant hemeoxygenase, and the anti-inflammatory collectin, surfactant protein-D, was decreased in the lungs after SM exposure[74]. Treatment of mice with half mustard, 2-chloroethyl ethyl sulfide (CEES) resulted in an increase in BAL protein, indicating the alveolar epithelial damage, within 3 d. Expression of inflammatory proteins, NOS, COX-2 and MCP-1, are implicated in tissue injury. These responses were

attenuated in mice lacking the p55 receptor for TNF-α. TNF-α mediates early antioxidant responses to lung toxicants and targeting TNF-α signaling may be useful in mitigating lung injury, inflammation and functional alterations induced by vesicants[75]. Also desensitization in β2-adrenergic receptors of the lungs occurs in CEES-induced lung injury[76]. Acute respiratory distress syndrome (ARDS) is one of the aspects of inflammatory lung disease induced by SM. Ceramides may play an important role in the development of ARDS by reducing cholinephosphotransferase enzyme[77].

Human studies

Acute phase

Acute human lung exposures to SM could be lethal in the short-term periods, otherwise at least leads to chronic devastating airways or pulmonary disorders[78]. In acute phase, two forms of respiratory tract involvement may be encountered. Upper airways dominancy was represented by pharyngeal, palatal and tracheal lesions and lower airways dominancy. The initial picture includes cough and hoarseness followed by nasal discharge and shortness of breath concomitant with skin eruptions. Increased airway inflammation caused epithelial necrosis, hemoptysis and production of viscous sputum containing necrotic debris. In such severe cases, pulmonary infection and lung gangrene are possible[79]. Acute pulmonary exposure to SM caused upper and lower respiratory tract damage via inflammatory and oxidative injury pathways[80,81]. Upper airways inflammation includes acute laryngopharyngitis, mucosal hyperemia and edema. Lower airway symptoms include shortness of breath and productive cough with mostly an obstructive pattern in spirometric studies[82]. The severe form of lower airways disease can be expressed as ARDS, with high mortality rate. Although tracheal necrosis may be detected a few weeks after exposure in bronchoscopic examination, chest X-rays are still normal. In this interval and before the initiation of chronic illness, therapeutic interventions may be of great benefits[8].

Chronic phase

In chronic phase, COPD induced by SM described as mustard lung is specific and to some extent different from COPD resulted from other well-known causes. Both oxidative stress and apoptosis are known mechanisms, more involved in pathogenesis of mustard lung compared with COPD[83]. Nonetheless, some researchers have described the severity of chronic pulmonary involvement as stages I–IV using pulmonary symptoms (chronic cough, sputum, hemoptysis, dyspnea) and signs (crackles, rales, wheezing), based on the Global Initiative for chronic Obstructive Lung Disease classification[84,85]. Stage I: a large survey has shown that 10 years after a single, heavy exposure to SM in 197 veterans may cause chronic pulmonary complications, including asthma (10.65%), chronic bronchitis (58.88%), bronchiectasis (8.62%), airway narrowing due to blistering or granulation tissue (9.64%) and pulmonary fibrosis (12.18%)[86]. Stage II: after 15 years of exposure, different degrees of localized or diffused tracheal, tracheobronchial, glottis and subglottic stenosis may occur[87]. Stage III: an airway hypersensitivity to inhaled methacholine or cold air as the most characteristic feature of asthma, known as an indicator of irreversible airway changes and correlates to decreased forced expiratory volumes in 1 s (FEV1) value, encountered in most chemical warfare victims[88,89]. Stage IV: the last step is progressive deterioration of the lung tissues known as ''mustard lung'' evolving nearly 20 years after exposure[90].

Concerning the clinical problems in chronic phase, a cross-sectional study on 134 veterans showed that respiratory complications gradually increased over time[91]. Relative risks factors for developed long-term pulmonary complications were higher with increasing age and those who did not use masks[92]. In a large group of SM-exposed patients, wheezing was the most common pulmonary finding accompanied with rhonchi that were significantly more frequent in severely intoxicated patients[93]. Moderate or severe exposure to SM showed an equal risk of late pulmonary complication, whereas mild exposure has lesser risk[94].

Although Spirometry is a valuable diagnostic tool for the evaluation of pulmonary impairment during regular follow-ups, arterial blood gas and high-resolution CT (HRCT) of the chest are more objective for evaluation of the severity and for diagnosis of the respiratory complications[95]. In 479 Kurdish-exposed patients without receiving effective initial treatment, abnormal findings in spirometry (15.2%) and CT (46.6%) were not different between the patients with or without a history of blistering. Also respiratory symptoms (dyspnea, cough and sputum production) were more common and FEV1 were lower in blistering group[96]. In a large group of patients, spirometry revealed an obstructive pattern in all patients and pulmonary function test (PFT) revealed mostly normal and restrictive patterns[97]. HRCT can be a useful method for differentiating mustard lung from resistant asthma and lung injuries due to cigarette smoking. Parenchymal involvements are more frequent in chemical-induced injury and asthma, whereas airway involvements are more frequent in smokers[98]. Although HRCT in inspiration is normal in most of the affected patients, expiratory HRCT showed patchy air trapping as the most common finding, which is suggestive of small airway diseases such as bronchiolitis obliterans[99] (Figure 4). In the HRCT of a chronically exposed group, the most frequent findings were bronchial wall thickening, air trapping, bronchiectasis, and mosaic parenchymal attenuation, irregular and dilated major airways and interlobular septal wall thickening[100]. Bronchiolitis obliterans is the main underlying respiratory consequence and may relate to host factors rather than to severity of early symptoms[94]. Bronchiolitis, or bronchiolectasis and mucus stasis that are consistent with more proximal airways are not solely dependent on the severity of exposure[101]. Air trapping in small airways involvement (suggestive of bronchiolitis obliterans) and tracheobronchomalacia in large airways, both as long-term sequelae, have similar underlying process[102]. Serum high-sensitivity CRP level may be used as a marker for the severity of COPD in patients with SM poisoning[103]. Serum angiotensin-converting enzyme might influence the lung inflammatory and fibrotic responses in late phase of toxicity[104]. Serum IL-6 increased in patients with SM poisoning and COPD, and may have a direct association with airflow limitation[105]. In a long-term SM-induced

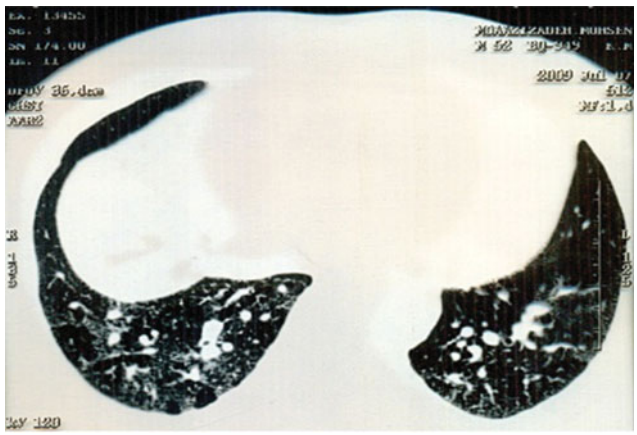

Figure 4. Expiratory HRCT of the chest in a patient with chronic SM-induced lung injuries. Patchwork appearances of numerous well-defined areas of decreased attenuation (dark areas) in both lungs representing air trapping. (By courtesy of Janbazan Medical and Engineering Research Center.).

pulmonary involvement, serum IL-6 was associated with wheezing and CRP was associated with wheezing and rales, but serum levels of IL-6 and IL-8 do not reflect the degree of severity of pulmonary involvement[85]. In BAL fluid of exposed patients, apolipoprotein A1 and S100 calcium-binding protein A8, and also a significant increase in vitamin D binding protein isoforms, haptoglobin isoforms and fibrinogen, and significant decreases in calcyphosine, surfactant protein A, and transthyretin were detected. These findings may be helpful in improving current concepts and monitoring of such complex illness[106]. Higher numbers of neutrophils, eosinophils, and higher concentrations of IL-8, granulocyte colony stimulating factor (G-CSF), GM-CSF[107], MCP-1, macrophage inflammatory protein-1α and β[108], IL-8 and TGF-β[109], RANTES (CCL5), eotaxin (CCL11) chemokines and IL-5 levels[110] in BAL fluid are associated with the development of pulmonary fibrosis in SM victims. The association of increased levels of BAL fluid cytokines CD4 lymphocytes in bronchiectasis[111], and CD8 T-cells and a lower CD4/CD8 ratio with lower percentage of diffusing capacity of the lung in pulmonary fibrosis have been proved[112]. TGF-β suppresses the inflammation and enhances the regeneration of tissue. TGF-β1 and TGF-β3 mRNAs were significantly increased in chemical gas-injured patients and play a protective role by improving airway remodeling and lung homeostasis in bronchiolitis obliterans[113]. A positive correlation has been reported between the severity of ocular and pulmonary involvements in a group of patients with severe mustard lung[14].

Skin complications

Animal and *in vitro* studies

In acute phase following cutaneous exposure in hairless guinea-pigs, erythema appears by 6 h and edema is seen within 24 h post exposure. In delayed phase, epidermal regeneration with hyperplasia and formation of granulation tissue in the dermis with the loss of hair follicles and glandular structures are encountered[114]. Hair abnormalities and loss even in the absence of obvious skin lesions or skin damage could be seen in guinea pigs exposed to low doses of CEES[115]. The trypsin with high protease activity is the most implicated enzyme in dermo-epidermal separation and blistering effect of SM[116]. The mechanism of vesication from SM and other vesicants such as cantharidin and Lewisite refers to inhibitory effects of one or more protein (serine/threonine) phosphatases in tissue cytosol *in vitro*, due to the concentration of TDG (the hydrolysis product of SM), rather than to the concentration of SM itself[117].

Most animal models, however, do not form the large fluid-filled blisters observed in humans[118]. Extents of injury are closely related to the exposure duration. Histological findings are severe edema, infiltration of inflammatory cells, damage to basal cells and vesication. By 2 weeks, impaired basement membrane and epithelial hyperplasia are observed. Prostaglandin (PGE) content and MMP-9 activity increased at 2 h post-exposure. PGE returned to baseline levels within 24 h, whereas MMP-9 remained elevated at least up to 48 h[119]. Pro and active types of MMP-2 and MMP-9 are significantly increased at 2 weeks post high-dose SM skin exposure[114]. SM induces keratin aggregation in keratinocytes. These aggregates begin to form within 15 min after SM exposure[120]. SM induced a dose-related loss of NAD^+ in endothelial cells and keratinocytes. Pathogenic events necessary for SM-induced vesication at higher vesicating doses of SM may depend on NAD loss with PARP activation and subsequent ATP-dependent effects on microfilament architecture. Vesication developing in lower concentrations of SM occurs by mechanisms other than cellular ATP (e.g. apoptosis and direct SM-mediated damage to integrins and the basement membrane). Exposure to lower concentrations of SM caused dramatic changes in keratinocyte morphology and microfilament architecture within 2–3 h. whereas higher concentrations significantly reduced keratinocyte adherence as early as 3 h after exposure[121]. Precise plane of epidermal–dermal junction separation in the HD-treated skin occurred beneath the hemidesmosomes within the upper portion of the lamina lucida[122]. The main proinflammatory mediators involved in SM-induced skin injury in a weanling pig model are increased transcriptional activity of IL-8, IL-6, IL-1β, and MMP-9 and TNF-α. IL-8 up-regulation encountered at 24, 48, and 72 h post exposure[123]. Human skin exposure to CEES leads to dermal and epidermal separation, nuclear chromatin condensation, spinous processes retraction, tonofibrillar collapse, cytoplasmic vacuolization, blebbing and loss of pseudobasement membrane integrity. Marked increased cellular and extracellular IL-1Ra suggests this cytokine as a tissue status marker and primary anti-inflammatory regulator in skin[124].

Laminin 5 is the main basement membrane protein affected acutely by HD exposure. The other basement membrane proteins showed no change or inconsistent changes[125]. Topical CEES treatment at high doses in mice skin caused a significant dose-dependent skin edema. Histopathological evaluation revealed increases in epidermal and dermal thickness, number of pyknotic basal keratinocytes, dermal capillaries, neutrophils, macrophages, mast cells, and desquamation of epidermis, epidermal cell apoptosis and basal cell proliferation. Following an increase in the

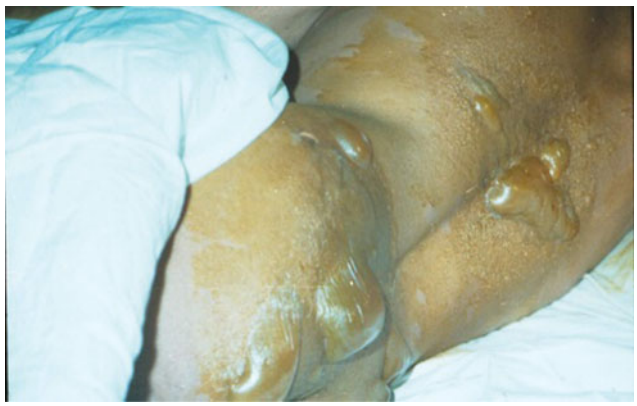

Figure 5. Acute cutaneous lesions induced by SM. Multiple large cutaneous bullous lesions over the dorsal surfaces of a patient few days after heavy exposure to SM vapor. (By courtesy of Seyed Naser Emadi, MD.).

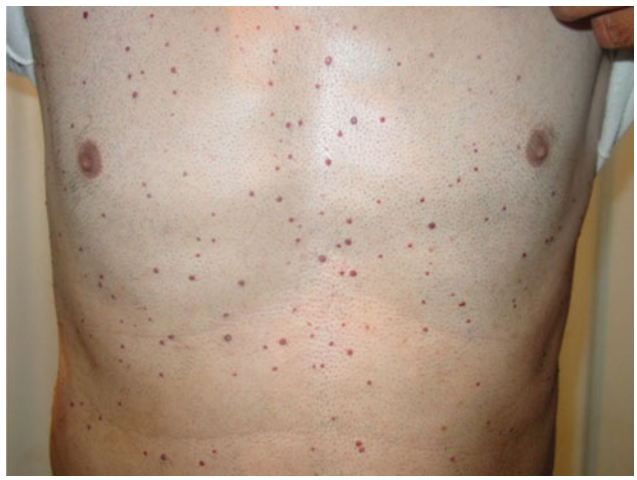

Figure 6. Chronic cutaneous lesions induced by SM. Multiple cherry angiomas over the trunk areas of a middle-aged man more than two decades after SM exposure. (By courtesy of Seyed Naser Emadi, MD.).

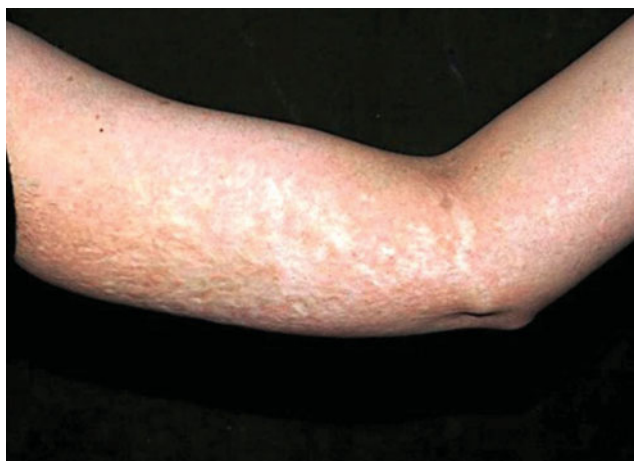

Figure 7. Chronic cutaneous lesions induced by SM. Left arm of a middle-aged lady. Several years after exposure, atrophic areas of skin scars are appeared at the site of primary bulla with irregular margins and wide depigmented patch at the center of the scars. (By courtesy of Professor Mohammad Ghassemi Broumand.).

mast cells, myeloperoxidase activity with neutrophil infiltration peaked at 24 h post exposure[126]. HD alkylates adhesive macromolecules of the basement membrane zone and inhibits their cell adhesive activity and disrupts the epidermal–dermal junction at the lamina lucida of the basement membrane in the process of HD-induced vesication 20 h after incubation. Sodium thiosulfate and cysteine prevented the cross-linking of basement membrane components subunits by HD[127]. Dermal inflammatory lesions and healing process in rabbits exposed to SM develops in 2–6 d, respectively. Hair follicles are the main source of new epithelial cells after the covering epithelium has been destroyed and GRO is probably a major autocrine–paracrine stimulus for such repair[128].

Human studies

Acute phase

In acute phase, various cutaneous lesions appeared in more than 90% of the patients exposed to SM. Absorption of SM through the human skin approximates up to 20% during the exposure time. Most of the agent (up to 70%) accumulates in the epidermis and the remainder penetrates deeper into the basement membrane and in the dermis. The liquid form can gradually evaporate and penetrate through the clothing and the warm temperature accelerates this condition. Acute cutaneous symptoms include itching and burning, whereas cutaneous clinical findings in this phase includes erythema or painless sunburn, bulla, hypo- and hyperpigmentation in both exposed and unexposed areas (Figure 5). The acute skin lesions gradually resolve to chronic delayed lesions by time with an obscure interval[129].

Chronic phase

Contact with SM agent can increase trans-epidermal water loss through the function alteration of stratum corneum as a barrier to water loss, several years after exposure[130]. In a case control study, exposed group including 362 patients showed significantly higher frequency of itching burning, xerosis, hyperpigmentation and cherry angioma[131]. In a large survey, among 800 Iranian exposed males, long-term skin lesions were seen in most of the patients and categorized into three types: (1) nonspecific lesions (93.4%) in which frequency of eczema (limbs), seborrheic dermatitis (scalp, face, chest), psoriasis (limbs), urticaria (diffuse), vitiligo (hand, scalp, face) and tinea versicolor (trunk, neck, upper limbs) were significantly higher than Iranian National Health Survey; (2) skin scars (5.5%) with irregular margins, pigmentary (salt and pepper appearance) and vascular changes (telangiectasia, cherry angioam), atrophic and hypertrophic reticular areas with islands of normal appearing skins; and (3) malignant cutaneous neoplasms (face scalp, limbs) with higher frequency (1.1%) than Iranian National Health survey (0.01%) including basal cell carcinoma (five cases), squamous cell carcinoma (one case), Bowen disease(one case), dermatofibrosarcoma protuberans (one case) and tumoral mycosis fungoides (one case) most of which were developed on old SM-induced skin scars[132] (Figures 6 and 7). In chronic phase, pathological skin changes include mild epidermal atrophy, mild papillomatosis and acanthosis along with patchy pigmentation in the basal layers, excess collagen deposition in dermis area with fibrosis and sclerosis, and atrophy of dermal appendices such as pilosebaceous units[132,133] (Figure 8). Recently, a causative correlation between exposure

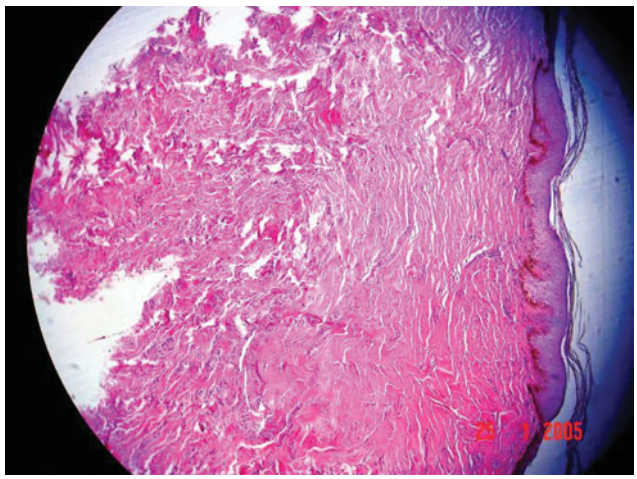

Figure 8. Chronic pathological skin changes induced by SM. Chronic pathological skin changes induced by SM exposure in groin area. Mild epidermal atrophy, mild papillomatosis and acanthosis along with patchy pigmentation in the basal layers, excess collagen deposition in dermis area with fibrosis and sclerosis and atrophy of dermal appendices,

to SM and induction of T-cell lymphoma/Sezary syndrome has been reported in the literature[134]. Also, based on clinical presentations such as skin atrophy, pigmentation and vascular changes on genitalia area supported by histopathological findings in hand lesions, including persistent pigmentation and skin appendix damages, a diagnosis of ''SM-induced poikiloderma'' has been suggested[135]. Comparing chronic cutaneous lesions induced by SM and nerve agents revealed that SM-exposed group were more involved to skin scars, intertrigo, xerosis, cherry angioma, hyper pigmentation, pilar keratosis, poikiloderma, and malignant lesions, whereas nerve agents group were more experienced to psychocutaneous disorders such as skin acne, seborrheic dermatitis and tinea versicolor[136]. The levels of SM exposure experienced by World War II veterans, which were sufficient to cause skin reactions, were not associated with any increased risk of cause-specific mortality[137]. In the study of Davoudi et al. performed on 310 SM-exposed patients, although xerosis was the most common complaint, but among the different skin areas (forehead, suprasternal, palm and back of the hands), the sebum was higher only in the forehead skin, without substantial effect on skin elasticity[138]. It is not yet clear whether the evolution of malignant cutaneous neoplasms on skin scars is directly related to SM effects or indirectly via DNA changes[1]. In 40 male subjects with delayed SM-induced skin lesions, except for the hematocrit and C4 levels, hematological and immunological parameters revealed no significant correlation with the severity grades of cutaneous complications[139].

Other organ complications

Gastrointestinal and liver complications

Animal and in vitro *studies*

Topically applied SM is hepatotoxic and caused disturbances in antigen-presenting, cell adhesion molecules, cytokine, cytokine receptor metabolism, fatty acid metabolism, GSH metabolism and cell cycle signaling pathway genes in mouse liver[140]. It also produces severe steatosis and significant rise in the levels of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT). The liver injury reached to peak on the third day[141]. In female mice treated by both inhalation and dermal exposure, liver was the most affected organ followed by spleen, thymus and lung[142]. Toxic effects of SM caused granulovacuolar degeneration with perinuclear clumping of the cytoplasm of hepatocytes by day 7. Maximum toxicity of SM reported in liver and kidneys[143].

Human studies

In 90 exposed patients, frequency of endoscopic and pathological esophagitis was significantly higher than that of the normal controls[144].

Hematologic, bone marrow, lymphatic and spleen complications

Animal and in vitro *studies*

Concomitant exposure of murine macrophage cells to lipopolysaccharide (LPS) and HD induced protection against HD-induced cytotoxicity. LPS exerts its protective action against HD toxicity through the generation of TNF-α and may provide better understanding of the mechanism of cytoprotection[145]. In mice, intoxicated with high doses of SM, a marked decrease in the number of total spleen cells was observed 1 week after intoxication. B-lymphocytes were relatively more affected than T-lymphocytes[146]. In hairless guinea pigs that were subcutaneously exposed to SM, a significant decreased in leukocyte count on days 4, 5 and 6 followed by an initial elevation on day 1 was observed[147]. Dermal exposure to SM leads to immune activation, infiltration of CD4 (+) and CD8 (+) T-cells into the SM-exposed skin, delayed-type hypersensitivity response in distal tissues associated with splenomegaly, lymphadenopathy, and proliferation of cells in these tissues. These immunological responses may contribute to the long-term sequelae of SM toxicity[148]. A single sublethal dermal dose of SM in Balb/c mice can cause considerable dose-dependent progressive fall in body weight, relative weights of spleen, liver and peripheral lymph nodes, a significant reduction in the cellularity of the spleen and thymus and degenerative histological changes and an increase in adrenal weight, red blood cell count, packed cell volume and hemoglobin concentration during 5–7 d after exposure[149]. Extensive exposure to SM can destroy the immune system by suppression of bone marrow cells[150].

Human studies

Severe bone marrow suppression has been known as a fatal consequence of extensive SM intoxication since many years ago[151–153]. Bone marrow is very sensitive to SM and even has been introduced as the most susceptible tissue to HD[154]. Atypical lymphocytes in peripheral blood smears of chemical victims exposed to SM have been reported. Change in lymphocyte shape may be related to committed stem cell involvement. The mild increase in erythroid cells and hemoglobin concentration may be due to chronic obstructive

pulmonary disorder and other respiratory diseases in these patients[155]. In overall blood exams, exposed patients had significantly lower numbers of PLT, WBC and PMN but from clinical viewpoint, this difference is not valuable. Further, lymph cells in the exposed group were significantly higher suggested the probability of severe damage to bone marrow[156].

Cardiovascular complications

Animal and in vitro *studies*

SM challenge on vascular endothelial cells in concentrations of <or = 250 μM induced exclusively apoptosis in 30% of endothelial cells 5 h after trial. Concentrations > or = 500 μM caused apoptosis and necrosis to the same extent in 60–85% of all cells between 5 and 6 h after the experiment. Necrosis was accompanied by a significant (approximately 50%) depletion of intracellular ATP, whereas in apoptotic cells, ATP remained at the level similar to healthy cells[157]. In the case of effects of SM on contraction of vascular smooth muscles, calmodulin kinase II may be a candidate target molecule of SM in early stage contraction of vascular smooth muscle[158].

Human studies

In a large group of SM casualties, typical chest pain and exertional dyspnea was reported to be more frequent than normal population but no differences were reported in terms of cardiac signs[159]. Scintigraphic pattern of myocardial perfusion showed that the prevalence of ischemia and non-homogeneity of uptake and left/right ventricular enlargement in both visual and quantitative analyses were higher in the mustard-exposed patients. Cavity to myocardium ratio, as an established and validated measure of ejection fraction, was also significantly lower in the exposed patients. These findings could resemble either coronary artery disease or mild cardiomyopathic changes[160].

Fallahi et al. evaluated cardiac symptoms and signs in SM-exposed patients by physical examination. The prevalence of chest pain was higher in veterans than controls.

Metabolic and endocrine complications

Animal and in vitro *studies*

As skin contains maximum number of metabolically active and rapidly dividing cells, SM was more toxic through percutaneous route compared with oral or subcutaneous routes in producing lung, liver and spleen damages[161]. Dermally applied SM in mice induced lipid peroxidation (revealed by increased MDA levels) and GSH depletion in blood and liver[162]. Percutaneous administration of SM in mice caused a significant decrease in oxidative stress enzymes such as GSH peroxidase, GSH reductase and superoxide dismutase. Flavonoids are promising cytoprotectants against this toxic effect. The decrease in body weight induced by SM and the histological lesions in liver and spleen were also significantly protected by the flavonoids[163]. After a single dermal application of 1.0 LD50 SM in mice, a significant hyperglycemia was observed at the first 24 h after exposure. There was a corresponding decrease in liver glycogen content, with no alteration in glycogen content of brain, muscles and kidney. Blood pyruvate and lactate levels were not appreciably altered[164].

Human studies

Total counts for RBC, MCV and total serum bilirubin were significantly higher in more severe intoxicated patients, but there was no statistically significant difference in direct bilirubin, SGOT, SGPT, ALP, hemoglobin, hematocrit, MCH and MCHC between exposed and controls[165]. In 286 male veterans with severe SM-induced pulmonary complications, significant decrease of serum albumin and albumin to globulin ratio and serum basal and salt-stimulated paraxonase activity were reported. Moreover, these decreases were in parallel with severity pulmonary complication[22].

Ear, nose and throat complications

Animal and in vitro *studies*

Hamster cheek pouches exposed to an analogs of SM, 2-CEES (or half mustard) showed greatest amount of structural alteration in the submucosal and muscle layers tissue 24 h later confirmed with OCT images and routine light microscope[166]. In animals, inhalation of SM only by nose resulted in severe nasal epithelial degeneration and minimal lung injury, whereas intratracheal inhalation of SM vapors resulted in homogeneous lung injury with no nasal degeneration[167].

Human studies

SM inhalation results in early and late toxic effects in ear, nose and throat systems. Fiber optic laryngobronchoscopy of the larynx in a group of 50 male Iranian veterans showed different degrees of dysphonia including harshness and hoarseness, supraglottic and subglottic regions inflammation and hyper function of the false vocal cords[168]. In a group of 43 male Iranian veterans, carcinomas of the thyroid and nasopharynx were seen in three of the patients[169].

Neurologic complications

Animal and in vitro *studies*

Historically, Guillain–Barre syndrome following exposure to SM in animal studies has been reported[170]. Chicken eggs injected with SM at a dose range below the threshold, on incubation days of 2 and 7 showed significant deficits in the intermedial part of the hyperstriatum ventrale (IMHV)-related imprinting behavior[171].

Human studies

Patients with long-term exposure to SM suffered from persistent damage to the afferent nerve system resulting in chronic neuropathic symptoms[172].

Psychological complications

Animal and in vitro *studies*

Chronic SM exposure of Swiss Albino mice for 12 weeks may have the potential to generate oxidative stress which may

trigger the release of cytochrome C as well as caspase-3 activation in neurons leading to cell death by apoptosis in a dose-dependent manner which may eventually be responsible for the disruption of cognitive functions such as rota rod, passive avoidance and water maze tests in mice[173].

Human studies

In a secret military test of SM on 363 volunteers, 32% showed full posttraumatic stress disorder (PTSD) with poorer physical health, a higher likelihood of several chronic illnesses and health-related disability, greater functional impairment, and higher likelihood of health care use, and 10% showed partial PTSD[174]. Lower lung dysfunctions as assessed by the St George Respiratory Questionnaire worsen the quality of life in patients with chemical warfare-induced COPD[175]. Short Form Health Survey (SF-36) showed that the lowest scores were found in chemical warfare victims who had ophthalmologic problems. Logistic regression analysis indicated that those who did not participate in sport activities suffer from a poorer physical health. Poor mental health was associated with longer time since exposure and lower education[176]. Dermatology Life Quality Index test in veterans suffering from severe itching showed a significantly poorer quality of life than do patients with milder symptoms[177]. A positive relationship has been found between higher physical activity and the serum levels of anti-inflammatory cytokine IL-10. This finding indicates a need to encourage a more active lifestyle among the SM exposed subjects who have various inflammatory complications[178]. Physical activity using Global Physical Activity Questionnaire (GPAQ) showed a significantly lower scores in terms of total physical activity MET (metabolic equivalent), total physical activity (min/week) and total transport-related physical activity (min/week) in BMI $>30\,kg/m^2$. In addition, total work-related physical activity was significantly lower with BMI $<25\,kg/m^2$. A significant correlation was reported between the increase of body weight and the reduction of total physical activity and MET in exposed group[179]. Physical activity status using GPAQ showed a significant decrease of physical activity in all stages of the test (pre-adoption and post-adoption stages) in exposed patients[180]. Psychological health status using Symptom Check List 90-Revised (SCL90-R) questionnaire impaired in those exposed who suffer from severe organs complications[181].

Reproductive complications

Animal and in vitro *studies*

In female rats challenged with SM caused early fetal resorptions and pre-implantation losses and decrease in total live embryo implants, and in male rats, a significant increase in the percentage of abnormal sperm was detected at a dose of 0.50 mg/kg. The timing of dominant lethal effects is consistent with an effect during the post-meiotic stages of spermatogenesis, possibly involving the generally sensitive spermatids[182].

Human studies

In about half of the patients who were infertile years after SM exposure, Azoospermia and severe oligospermia were diagnosed. An elevated plasma follicle-stimulating hormone (FSH) and normal levels of plasma luteinizing hormone and testosterone concentrations were found. Testicles biopsy showed selective atrophy of the germinal epithelium[183]. In a relatively large group of SM-intoxicated patients, male factor infertility was significantly different between married exposed and unexposed casualties. All semen indices declined over a period of 15 years among the exposed group. Furthermore, all indices with the exception of sperm motility were significantly lower in exposed than in the unexposed. The FSH levels were significantly higher in the infertile than in the fertile exposed men. Histopathology of the testis of the azoospermic men showed complete absence of spermatogenesis with only Sertoli cells in the seminiferous tubules. Chronic toxicity of SM may be gonadotoxic with permanent effects. Probably, the most susceptible gonadal cells to SM are the germ cells[184]. Although SM exposure was associated with abnormal semen parameters; exposure to depleted uranium had no effect on semen characteristics[185]. In children and teenagers, genital manifestations were less frequent than systemic problems[186].

Urinary tract complications

Animal and in vitro *studies*

Toxic effects of SM caused granulovacuolar degeneration with perinuclear clumping of the cytoplasm of renal parenchymal cells by the day 7. Renal lesions were characterized by congestion and hemorrhage[143].

Human studies

In a report on 289 Iranian male veterans who had been exposed to high doses of SM, history of urinary calculi, recurrent urinary tract infections, benign prostatic hyperplasia and renal failure were in order of frequency. None had experienced urogenital malignancies. Neither recurrent urinary tract infections nor urinary calculi were significantly associated with age, medications and their doses, or SM-induced late complications in other organs[187].

Musculoskeletal complications

Animal and in vitro *studies*

Single dermal application of 1.0 LD50 SM in mice caused a significant hyperglycemia within the first 24 h post exposure in correspondence with decrease in liver glycogen content, without any alteration in glycogen depletion of the brain, kidney and muscles[164].

Human studies

In SM-exposed patients, the chief musculoskeletal complaint was lower extremity pain; the most frequent physical finding was widespread tenderness and the most frequent clinical diagnosis was psychogenic pain. Among laboratory tests, only anti-nuclear antibody (ANA) titer was significantly higher in more severe intoxicated patients. The most frequent rheumatologic complications were degenerative diseases of joints and spine. There were more rheumatoid arthritis patients among

the victims. Higher ANA titers may indicate some autoimmune disorders as late rheumatic complications of SM[188].

Teratogenicity

Animal and in vitro *studies*

SM in female mice causes down-regulation of anti-inflammatory cytokine and receptor gene expression and up-regulates pro-inflammatory genes TNF-α and TNF receptors, both from day 1 to day 3, activates the cascade of events in the signal transduction pathway and promotes irreversible double-strand DNA breaks in chromosomal DNA, which leads to cell death between the days 1 and 7[189]. DNA inter-strand cross-links that may act as progenitors of malignant cells are induced by SM[190].

Human studies

On the basis of rating of International Agency for Research on Cancer, SM is categorized in group number 1 (agents that are carcinogenic to humans)[191]. As SM is an alkylating substance, it is conceivable that the risk of developing cancer may be increased, as observed in people who were involved with the production of SM. Also, transient significantly increased sister chromatid exchange rates have been found in fishermen exposed to SM[192]. It is well documented that long-term exposure to SM can cause human lung cancer, but there has not been strong and definitive evidence for only short-term and acute, single, high-dose exposure until now[193]. A relatively early age of lung cancer onset in SM victims, particularly in non-smoking population has been described. A single exposure may increase the risk of lung cancer development in some individuals[194]. Carcinoma of the larynx and trachea were significantly higher in men and women workers of the SM manufacturer company compared with the community expected numbers[195]. Chronic myelocytic leukemia has also been reported in such workers. High incidence of chromosome abnormality and sister chromatid exchange rate as well as cytogenetic changes was even equivalent to those of atomic bomb survivors exposed at 1.2 km from the site of explosion[196]. Inhalation of small amounts of SM damaged somatic cell genes, resulting in carcinogenesis[197]. Previous screening studies confirmed that exposure to SM can cause cancers of the upper respiratory tract and some evidence that it can cause lung cancer and non-malignant respiratory disease[198]. Long-term follow-up of former workers in the poisonous gas factory showed that SM exposure transforms the age scale for developing lung cancer in younger ages[199]. Occupational exposure to specific chemicals including SM has increased the risk of renal cell carcinoma in the males[200].

Divers and scattered human case report studies

In a nearly recent accident in workers exposed to SM, exception of those common complications of SM, one case of Barrett's esophagus and another of oral metaplasia has been reported[201]. A repeated gastrointestinal malignancy has been reported in a case of SM gas injured patient[202]. The resected stomach from a 58-year-old male patient showed moderately differentiated tubular adenocarcinoma with invasion reaching the submucosa. Widespread metastases of lymph nodes including superior mesenteric and para-aortic area were noted in a worker of SM factory[203]. An unusual case of a chemical victim presenting with characteristic mustard scar leading to stenosis of the external meatus has previously been discussed[204].

Management

Animal and *in vitro* studies

For acute ocular injuries induced by SM in rabbits, installation of local steroids (DEX, four times daily/1 week) starting within 1 h post exposure caused significant amelioration of the inflammatory response during the first 24 h without any effect on corneal erosions. The effect of this initial treatment is long lasting and significantly reduces and postponed the acute and chronic corneal edema up to 2 months. Although the treatment diminished and postponed the delayed corneal NV, it did not prevent its appearance. Also the ameliorating effects of DEX on corneal thickness and NV for symptomatic relive, starting at 2 weeks after exposure are significant and long lasting as far as the treatment continues. Amniotic membrane transplantation in delayed keratitis at 2 weeks after exposure showed a slight, non-significant improvement in corneal opacity and edema[54].

Although no specific antidote currently exists for SM exposure, recent studies using the SM analog CEES in animals have focused on the ability of antioxidants to prevent toxicity[205]. Inhalation or percutaneous exposure of SM in Swiss albino female mice caused oxidative damage to liver and lung tissues and resultant decrease in GSH and increase in MDA. Antioxidants administrated immediately and once daily for 2 d after SM exposure could enhance survival time through protection of the liver and lung tissues from oxidative stress and reduction in accumulation of purine metabolites in blood[206]. As the neutrophils are the most common inflammatory cells (up to 88%) in BAL fluid[207], *N*-acetylcysteine (NAC) decreased inflammatory response through decreasing neutrophil counts in BAL fluids[208]. NAC-containing liposome reduced the lung permeability index and the appearance of proinflammatory mediators in BAL fluids to the baseline levels and suppresses progressive fibrosis[209]. Given the data supporting efficacy of NAC against HD effects with low toxicity and no needs for regulatory controls, daily oral administration of the maximum safe dose of NAC to personnel entering combat zones has been suggested[150].

CEES inhibit nitric oxide (NO) production in LPS-stimulated macrophages by decreasing iNOS. Since NO acts as an antioxidant, the CEES-induced down-regulation of iNOS in LPS-stimulated macrophages could elevate oxidative stress[210]. Although NAC reduced oxidative stress in LPS-stimulated macrophages treated with CEES, it did not reverse CEES-induced loss of NO production. NAC and polymyxin B were found to help prevent CEES toxicity in LPS-treated macrophages[211]. Antioxidant liposomes containing both NAC and vitamin E are an effective antidote against CEES-induced lung injury[212]. Cellular GSH content have therapeutic implications in particular for the protection of lungs after inhalation exposure to HD vapor[15]. Nifedipine, by restoration

of GSH levels and prevention of lipid peroxidation in liver significantly increased mean survival time in SM challenged male albino mice[213]. Pretreatment of mice with GSH before topical application of CEES resulted in significant protection against CEES-induced skin edema, apoptotic cell death and myeloperoxidase activity[214]. Pyrimethamine and cimetidine as immunomodulating agents are effective in augmenting immune responses after SM-induced immunosuppression through augmenting antibody titers, enhancing delayed type hypersensitivity, restoring splenic follicles and increasing macrophage numbers and phagocytic activity[215]. Macrolide antibiotics (azithromycin, clarithromycin, erythromycin and roxithromycin) may lead to improved clearance of apoptotic material in the airway by improving the degenerated chemotactic and phagocytotic functions of monocytes and ultimately results in reduced airway inflammation and injury caused by SM inhalation[216]. In guinea pigs, lung surfactant damages induced by inhalation of SM in saline could be managed by a combined exogenous surfactant, anti-inflammatory drugs and broncholytics[217]. A combination of dexamethasone (significantly reduced IL-1 and IL-6 in BAL fluid) and vitamin E (an antioxidant with anti-inflammatory effects) could be used for protection against chemical-induced lung injury[218]. In acute skin exposure, sodium hypochlorite (0.5% and 2.5% solutions), calcium hypochlorite (0.5% and 2.5% solutions) and sterile water could be used with equal decontamination efficacy[219]. Simply an early and noninvasive act of cooling on HD-exposed skin may provide a simple route for reducing the severity of HD-induced cutaneous lesions with permanent effects[220]. Topical treatment with povidone-iodine (PI) or iodine ointment significantly reduced skin damage induced by SM. The combination of anti-inflammatory agents and iodine enhances these effects[221]. Iodine preparation (PI) combined with steroidal and non-steroidal anti-inflammatory agents that function as an antidote against skin lesions induced by SM in animal models[222]. As TNF-α induction was shown to be associated with reactive oxygen species (ROS) production, the combination of local application of iodine and systemic administration of anti-TNF-α antibodies via intravenous injection might constitute a new approach for treatment of SM-induced skin lesions[223]. Cyclooxygenase-1 and -2 (COX-1, COX-2), play important roles in SM-induced acute skin toxicity. COX-2 participates in the early stages and COX-1 might exert some protective function against this chemical insult[224]. SM vesicants target cytochrome P450 reductase and that this effect may be an important mechanism mediating oxidative stress and lung injury[225]. 1 -α, 25(OH) 2D3 could be an alternative treatment for cutaneous inflammation disorders caused by SM because of its ability to suppress inflammatory mediators and enhanced cell proliferation in human skin cells[226]. Epithelial cell adhesion is under control of the local cytotransmitter acetylcholine working through the muscarinic and nicotinic receptor. These findings support a hypothesis that pharmacologic protection from the vesicating action of HD can be achieved using cholinergic drugs[227]. In an experience on SM exposed abdominal skins of weanling pigs, different depths (100–400 microns) of laser debridement on granulation tissues formed 48 h after exposure, showed complete re-epithelialization in 7 d. In general, with different post laser dressings, more superficial laser debridement (100 microns) and less damage to basement membrane provide better wound healing than deeper debridement (400 micron) with respect to early re-epithelialization, cosmetic appearance, functional restoration and structural integrity[228]. For bone marrow supression, G-CSF, a hematopoietic growth factor has been introduced as a treatment protocol because of its safety and absence of serious side effects[229].

Human studies

Systemic management in acute phase

Despite many years from discovery and use of SM in different wars or accidents no serious antidote still existed. Therefore, preventive modalities such as suitable masks and clothing and goggles in danger zones are highly advised[62]. Effective initial management and intervention following acute SM exposure can only be accomplished by rapid decontamination followed by palliative treatment of symptoms[230]. Systemic intoxication could be managed by the administration of sodium thiosulphate (500 mg/kg) with or without other adjunctive medications, such as steroids, antihistamines and vitamins[16,231]. Medical attention is primarily focused on the vital target organs including lesions of the eyes, respiratory tract, skin and gastrointestinal systems, followed by bone marrow depression[232].

Ocular managements

Acute phase. Emergent washing of the eye for at least 10–15 min in any patients suspected to exposure, with copious taped water or other available useful solutions (such as Ringer or normal saline, sodium bicarbonate1.5%, and magnesium sulfate) is mandatory[58]. In cases with mild conjunctival involvement, management included conservative measures, e.g. preservative free artificial tears, lubricants and topical steroids[16,58]. In this phase for corneal involvement, regular daily ocular surface irrigation, artificial tears, bandage contact lenses, midriatics, antibiotic and anti-glaucoma medications should be considered based on the findings encountered[10,58,233]. However, use of any topical steroids, eye ointments or lubricants and ocular patch are better to be avoided due to their possible hazards[16,58]. In the cases of continuous ocular inflammations, like other types of chemical ocular burns, one may use amniotic membrane transplantation to protect the cornea against progressive melting and perforation[234].

Chronic phase. Ocular managements in chronic phase for milder cases include conservative medical management such as tear substitutes, short-term topical or systemic steroids especially in exacerbation of inflammation, bandage, high oxygen permeable soft contact lenses and punctal plugs or punctal cauterization of one or both puncti[63]. For more severe cases such as persistent epithelial defects and corneal involvement, surgical interventions, including lateral or medial tarsorrhaphies, amniotic membrane transplantation, lamellar or penetrating keratoplasty, stem cell transplantation and lamellar or penetrating keratoplasty may employed based on the severity and the type of involvement[63,235].

Respiratory managements

Acute phase. Respiratory distress should be managed immediately by administration of oxygen and steam inhalation and bronchodilators, mucolytic, or other proper medications such as systemic steroids and antibiotics. Immediate laryngeal intubation in severe respiratory distress and assisted ventilation are mandatory in life-threatening pulmonary insufficiency[56].

Chronic phase. Short-term intravenous or oral corticosteroid therapy has a significant effect on lung function of almost 50% of patients with SM-induced chronic bronchitis in exacerbation occasions[236]. Inflammation and pulmonary fibrosis processes may be progressive in SM-exposed patients, so IFN-γ has been suggested for these patients' treatment[237]. It is reported that treatment with a combination of 200 μg of IFN-γ-1 b (given three times per week subcutaneously) plus 7.5 mg of prednisolone (given once a day), for 6 months, was associated with an improvement in the lung function in SM-exposed patients with bronchiolitis[238]. However, there was no report on IFN-γ levels in SM injured patients. For clinical use of cytokines more investigations are needed to clarify the role of cytokines in late complications induced by SM. Inhaled corticosteroids and long-acting β2-agonists are reported as effective in the treatment of patients with chronic bronchiolitis following exposure to SM. However, a medium dose of fluticasone/salmeterol has the same effect on the airways reversibility, rather than a very high dose of beclomethasone with only the short-acting β-agonist[239]. Airway responsiveness to salbutamol increased in most subjects exposed to chemical warfare; this responsiveness correlated with airway caliber[240]. Four months administration of NAC (1800 mg daily) can improve clinical conditions and spirometric findings on SM-induced bronchiolitis obliterans syndrome[241]. Macrolide antibiotics decrease the production of proinflammatory cytokines and mediators and improved macrophage functions in clearance of airways from apoptotic material[216]. Despite such modalities, the problem still remains in majority of the patients and needs more investigations.

Skin management

Acute phase. Diversity of skin preparations halts to introduce an especial protocol effective for all types of skin lesions in acute or chronic phases. For extensive fluid loss from huge skin blisters or other parts of the body, fluid and electrolytes substitutions with proper solutions are needed. Skin management includes coverage of the exposed areas with solutions or ointments with antibacterial activities such as povidone iodine or silver sulfadiazine[56,62,242]. To ameliorate the skin damages, special barrier creams before exposure and absorbent powders such as calcium chloride, magnesium oxide, activated charcoal, and washout with warm water or other suitable solutions (such as hypochlorite 0.5%, calcium hypochlorite) after exposure have been recommended[16,243].

Chronic phase. For skin lesions topical pimecrolimus 1% was as effective as betamethasone cream 0.1% in controlling pruritus, burning sensation and skin dryness of SM-exposed patients[244]. A combination therapy with phenol and menthol 1% has significant therapeutic effects for SM-induced pruritus in chemical warfare-injured veterans, in comparison with the placebo[245]. In relieving SM-induced chronic pruritus, hydroxyzine 25 mg/d has equal results compared with doxepine 10 mg once daily; but greater than cetirizine 10 mg once a day[246].

Future treatment opportunities

Identifying ways to repair DNA may create enormous hope for the future treatment of these patients. Homologous recombination in DNA with nucleotide excision repair and non-homologous end joining are the major repair pathway protecting against acute SM toxicity[247]. Immunomodulatory interventions are another valuable prospect in these patients. Polymerase and MMP inhibitors, anti-inflammatory drugs, antioxidants and perhaps regulators of DNA damage repair are identified as promising approaches to minimize SM-induced organ damage and late effects[81]. NAD+ as a substrate, repairs the PARP-induced molecular damage. Melatonin with the ability of scavenging both oxygen and nitrogen-based reactants such as ONOO− and blocking transcriptional factors that are capable to induce pro-inflammatory cytokines play effective roles in ameliorating the acute and delayed effects of SM-induced tissues injuries[248]. Developing of stem cell transplantation techniques for revitalization of compromised vital organs such as the eyes, lungs, skin and etc. actually help to decrease the difficulties of management in these patients[14,66,249].

Discussion and conclusion

This review showed that most of the published SM-related articles, studies and researches in order of numbers are focused on the skin, lungs, eyes and systemic complications. Aside from the interest of researchers, the cause of such order of frequency may at least in part refer to the clinical presentations of each organ involvement. In this regard, clinical course of early painful and bizarre appearance of skin blisters, the vital importance and intermediate acceleration of respiratory distress, initial improving of ocular symptoms with far later visual impairment and infrequent or less important other systemic disorders, may influence on this interest.

Oxidative stress in exposed tissues included increased formation of ROS, production of lipid peroxidation and oxidized proteins derivatives, and increases in superoxide dismutase, catalase and glutathione-*S*-transferase. Drugs that neutralize the oxidative stress pathways may be appropriate candidates for reducing SM-induced tissue injuries[250]. Cytotoxic effects of HD could prevent by niacinamide and 3-aminobenzamide for up to 2 d[251].

Eye symptoms are slowly progressive; however a severe, rapid onset form of keratitis may develop in a number of patients after a latent period of 15–20 years. Most of the acute ocular problems heal during a few weeks after exposure, but rare regression of the signs and symptoms can occur up to more than 20 years after exposure, the so-called delayed keratitis that may lead to an unpredictable penetrating keratoplasty and blindness[62].

In the lung, following SM poisoning, DNA damage, apoptosis and autophagy are associated with inflammatory cell accumulation in the respiratory tract and increased expression of TNF-α and other proinflammatory cytokines, as well as reactive oxygen and nitrogen species. MMPs are also upregulated in the lungs after SM exposure, which are thought to contribute to the detachment of epithelial cells from basement membranes and disruption of the pulmonary epithelial barrier. The last specific therapeutic interventions include anti-inflammatory agents (e.g. steroids), antioxidants (e.g. tocopherols, melatonin, *N*-acetylcysteine, nitric oxide synthase inhibitors), protease inhibitors (e.g. doxycycline, aprotinin, ilomastat), surfactant replacement, and bronchodilators. Effective treatments may depend on the extent of lung injury and require a multi-faceted pharmacological approach[78]. Administration of liposomes containing reducing agents such as NAC, GSH and resveratrol at least 1 h after exposure, significantly reduced acute SM-induced lung injuries[252].

Skin damages induced by SM in the acute phase are characterized by edema, inflammation and basal keratinocyte cell layer death, whereas in chronic phase, xerosis, hypo or hyper pigmentation, scars, and even in rare cases, skin cancers have been discussed. Combination therapy with topical drugs and oral antihistamines, also iodine and recently anti-TNF-α antibodies, have introduced as effective medications for the management of skin lesions[253]. Also Baicalin, a bioactive flavonoid extracted from the roots of *Scutellaria* spp., has recently been introduced as a contractor against diverse molecular and biochemical abnormalities induced by SM dermatotoxicity[254]. Recent studies have focused on low molecular weight antioxidants. Melatonin, epigallocatechin gallate and flavone derivatives are among such suggestions[255].

In addition other potential effects in the longer term including the development of cancer, immunological and neuropsychiatric changes, and reproductive effects are being investigated. Finally, long-term social and economic effects of these illnesses on individuals and their families are now becoming apparent[90].

Recently, valuable and extensive review articles especially in the fields of non-classic manifestations and biological effects of SM have been added to the medical literatures that we refer the interested readers for more detailed information[256,257]. Such works reflect the increasing efforts of human being to conquer and overcome the unknown aspects of SM. We expect these advances in knowledge lead to development of new interventions in SM-related pathogenesis, or possibly at least reversing some of its adverse effects. In conclusion, during the last century and up to now, the scientists have realized that only minimizing SM-induced organ damages and late effects using modern medicine created nowadays seems the proper management of choice in these patients[80]. Stem cell transplantation for ocular[66], pulmonary[14] and skin lesions[249] induced by SM are of current treatment recommendations or proposed challenging interventions that may change the future therapeutic strategies in SM-exposed patients. Despite such developments, due to the complexity and pervasiveness of the toxicity, uncoordinated efforts seem to be less successful. Given the constant and multi-organ complications of SM and lack of a single highly effective antidote, a package of continuous multi-disciplinary team work approach for diagnostic and therapeutic purposes in specific and well-designed medical centers is recommended for these complex patients.

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Declaration of interest

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