REVIEW

Chronic health effects of sulphur mustard exposure with special reference to Iranian veterans

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The widespread use of sulphur mustard (SM) as an incapacitating chemical warfare agent in the past century has proved its long-lasting toxic effects. It may also be used as a chemical terrorist agent. Therefore, all health professionals should have sufficient knowledge and be prepared for any such chemical attack. SM exerts direct toxic effects on the eyes, skin, and respiratory tissue, with subsequent systemic action on the nervous, immunological, haematological, digestive, and reproductive systems. SM is an alkylating agent that affects DNA synthesis, and, thus, delayed complications have been seen since the First World War. Cases of malignancies in the target organs, particularly in haematopoietic, respiratory, and digestive systems, have been reported. Important delayed respiratory complications include chronic bronchitis, bronchiectasis, frequent bronchopneumonia, and pulmonary fibrosis, all of which tend to deteriorate with time. Severe dry skin, delayed keratitis, and reduction of natural killer cells with subsequent increased risk of infections and malignancies are also among the most distressing long-term consequences of SM intoxication. However, despite a lot of research over the past decades on Iranian veterans, there are still major gaps in the SM literature. Immunological and neurological dysfunction, as well as the relationship between SM exposure and mutagenicity, carcinogenicity, and teratogenicity are important fields that require further studies, particularly on Iranian veterans with chronic health effects of SM poisoning. There is also a paucity of information on the medical management of acute and delayed toxic effects of SM poisoning—a subject that greatly challenges health care specialists.

History

Synthesis

Sulphur mustard (SM) or mustard gas is bis(2-chloroethyl) sulphide and was first synthesised in 1822 by Despretz. Its vesicant properties were noted by Guthrie in 1860.[1] In 1886, Victor Meyer was the first to prepare pure SM by the reaction of thiodiglycol with phosphorus trichloride. Thiodiglycol was prepared by the reaction of 2-chloroethanol with potassium sulphide. Thiodiglycol can also be prepared by the American process, in which ethylene oxide reacts with hydrogen sulphide. SM was produced for use as a chemical warfare agent (CWA) by what is known as the Leveinstein process: the reaction of ethylene with sulphur dichloride before the First World War (WWI).[2]

Applications

SM has been the most widely used CWA in the past century. It was employed extensively in the WWI between 1914 and 1918. In spite of the Geneva Protocol in 1925 on the prohibition of CWA, SM was used by Italian troops in Ethiopia (1935–1936) and by Egyptian forces in Yemen (1963–1967). Recently, the greatest use of SM was by the Iraqi Army against Iranian soldiers and even civilians in Sardasht and Halabjah between 1983 and 1988, resulting in over 100,000 chemical casualties.[3]

Although there have been no substantiated reports of the use of SM by terrorist groups, the simplicity of its chemical synthesis does offer the potential for use by terrorists.

Type of exposure

Single

Most human cases of SM poisoning have occurred during armed conflicts and most accidents were a single exposure.[4]

Multiple

Multiple, low, SM exposure occurred occupationally and during the WWI and in the Iran–Iraq conflict.[4,5]

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Secondary

First-aid workers, nursing, and medical staff who were looking after SM casualties in the field clinics and hospitals during the Iraq–Iran war without proper personal physical protection have become intoxicated. Some of them are now suffering from the delayed toxic effects of SM and have disabilities of between 5 and 25%.[6]

Sub-clinical

Sub-clinical exposure to SM in some Iranian combatants induced delayed toxic effects. A study on 77 subjects, who were present in a contaminated area and had no acute symptoms or signs at the time of exposure, are now suffering from respiratory disorders such as bronchiectasis and bronchiolitis obliterans.[7]

Chronic

Chronic SM exposure is usually occupational. Some factory workers in Japan and in the UK were reported to have had SM poisoning and even malignancies due to SM.[8,9]

Routes of exposure

Inhalation

Inhalation is the major route of exposure that induces respiratory and systemic toxicity following absorption across the lung surface.[10]

Dermal

SM is a vesicant or blistering agent that has direct toxic effects on the skin producing erythema, blistering, epidermolysis, and necrosis. It is a lipid-soluble compound and thus can be readily absorbed across the skin.[2,11]

Ocular

The eyes are the most sensitive organs to SM. This marked susceptibility is attributable to several ocular features including the aqueous–mucous surface of the cornea and conjunctiva, as well as the high turnover rate and intense metabolic activity of the corneal epithelial cells.[5,12,13]

Oral

SM may also enter the body by oral ingestion. We observed a few Iranian combatants during the war who had ingested food contaminated by SM and who subsequently became intoxicated. They experienced nausea, vomiting, haematemesis, abdominal pain, and dyspnoea. SM may also be absorbed through the lower gastrointestinal tract.[14]

Injections

Injection is a very rare route of SM intoxication and has not been reported.

Toxicity

Supra-acute

Exposure to very high doses of SM in the field may induce convulsions and death in less than 1 h.[15,16] Such observations have not been reported during the Iraq–Iran war.

Acute

Acute toxic effects generally appear after variable periods of latency depending on the dose, mode of exposure, the environmental temperature, and probably on the individual.[2,17,18]

Sub-acute

Sub-acute exposure occurred during the Iran–Iraq war and in the workers in the SM munitions factories. However, this type of exposure may present as a mild, acute SM intoxication or as a complication in the respiratory tract or even as malignancy.[6]

Delayed

Delayed toxic effects of SM have been documented. The first report of delayed toxic effects in Iranian veterans was reported in 1986.[19] Several articles on the delayed toxic effects and complications of SM in Iranian veterans have been published since.[6,7,10,11,13]

Chronic

Several studies suggest that workers who were chemically exposed to mustard agents in the British and Japanese munitions factories developed chronic respiratory effects. In a cohort mortality study of 3500 workers at a manufacturing plant in England, a statistically significant increase in the number of deaths due to influenza, pneumonia, bronchitis, and asthma were reported. This was present even among those with fewer than 3 years of employment at the plant and so was not related to the duration of employment.[20]

A 25-year follow-up study of workers exposed to SM in a Japanese production plant revealed that more highly exposed workers had more chronic bronchitis and a slightly lower forced expiratory volume in one second/forced vital capacity (FEV1/FVC) ratio than either the less-exposed or an unexposed group of their co-workers.[9] In another study, Brown[8] reported a large number of employees who worked at the Huntsville Arsenal in Alabama. They were continuously exposed to the gas over long periods of time and developed bronchiectasis with progressive emphysema and narrow attenuated bronchioles.[8] Cancer of the respiratory system has also been associated with occupational exposure to SM and is discussed later in this article.

Toxicodynamics

Monofunctional mustards have one alkylating site and therefore can attack and break the DNA at specific nucleotides. Although SM reacts with RNA, proteins, and phospholipids, the consensus view is that a DNA alkylate, plays an important role in delayed toxic effects.[21,22] The major alkylating site of nucleic acids of mammalian origin is the nitrogen residue of guanine.[23] Cell death from DNA cross-linking is delayed until the cell replicates its DNA or undergoes division. At higher

cellular exposures, however, mechanisms other than DNA cross-linking become important and produce more rapid cell death. The acute damage to the cornea, mucous membranes, and skin seen after SM exposure is probably generated by one or more of these other mechanisms. One mechanism that may be involved in acute damage is nicotinamide adenine dinucleotide (NAD) depletion. Other potential mechanisms of cell death are related to rapid inactivation of sulphydryl-containing proteins and peptides, such as glutathione. These sulphydryl compounds are critical in maintaining the appropriate oxidation–reduction state of cellular components. Glutathione is also thought to be critical in reducing reactive oxygen species in the cell and preventing peroxidation and loss of membrane integrity.[24,25]

Toxicokinetics

SM is absorbed by inhalation, through the skin, or through the anterior surface of the eye. It may also be absorbed through the gastrointestinal tract following consumption of contaminated food. When delivered as liquid or vapour, the skin plays a very important role as a port of entry for SM. SM undergoes hydrolysis producing half-mustard and thiodiglycol, which is the major metabolite and is excreted in urine.

From the total mustard that penetrates, only 10–20% is fixed to macromolecules in skin. The remaining 80–90% is rapidly transported away from the site of absorption by the circulation.[26–28] In terminally ill cancer patients, 80–90% of the radioactivity of the injected ^{14}C-labelled SM disappeared after several minutes from the blood and was excreted mainly in the urine within 24 h.[29] SM is eliminated in a two-compartment model. The distribution of SM is quick with a long terminal half-life (5.56 min and 3.59 h). The volume of distribution at steady state (V_{dss}) is 74.41.[30] Whole body autographic studies with ^{35}S-labelled SM have shown that elevated radioactivity was detected in the nasal region, followed by the kidneys, liver, and intestines at all times studied after percutaneous or intravenous administration.[31] In humans, unhydrolysed SM can be present in brain and fat depots days after exposure.[32] Few data are available on biotransformation of SM in man. Two studies in rats revealed that conjugation with glutathione is more important than hydrolysis.[29,33] More recent investigations demonstrated that 60% of the dose is excreted within 24 h in the urine.[30]

Mutagenicity

There is no evidence for the mutagenicity of SM and no evidence of teratogenicity was found in rats treated with different doses of SM.[17,34]

Carcinogenicity

SM is classified as a carcinogen by the International Agency for Cancer Research. Human studies indicated a causal association between occupational exposure to SM and the excessive occurrence of respiratory cancer, skin cancer, and, possibly, leukaemia.[35] A significant excess of deaths (33 cases against 0.9 expected) due to respiratory cancer was found among former workers of the Japanese poison gas factory that operated from 1929 to 1945.[36] Similarly, highly significant excesses in cancer of the larynx, pharynx, and other upper respiratory sites were observed in former employees of a British plant that manufactured SM. A moderate, but still significantly higher mortality than normal population was also observed in lung cancer.[37] Gastric cancer, basal cell carcinoma, Bowen's disease, Bowen's carcinoma, and skin spinocellulare have all been reported following occupational exposure to mustard gas.[20,38,39]

Effects on reproductive system

A few studies are available regarding the reproductive effects of SM. Intravenous injection of SM in male mice results in damage to the testes, with inhibition of spermatogenesis.[40] Nevertheless, the damage is usually transient, because testicular recovery is observed at two weeks, with the formation of mature sperms four weeks after exposure. A two-generation study of rats indicated that exposure to SM at levels of 0.03, 0.1, and 0.4 mg/kg/day did not have any adverse effects on reproductive performance or the fertility of male or female rats throughout two consecutive generations, except for an altered sex ratio in the 0.4-mg/kg group.[34]

Target organs

Eyes

Eyes are the most sensitive organs to SM. The first symptoms of SM exposure are usually those on the eyes.[5,12,13]

Respiratory tract

Next to the eye lesions, the greatest discomfort produced by mustard gas results from irritation and toxicity of the respiratory system. Respiratory effects occur in a dose-dependent manner from the nasal mucosa to the terminal bronchioles[5,10,41]

Dermis

Direct toxic effects of SM on the skin are the main apparent effects that lead to it being called a vesicant or blistering agent. A German medical toxicologist and an Iranian medical toxicologist (first author) classified the cutaneous mustard gas lesions as follows:

- erythematous form;
- pigmentary exfoliation;
- superficial vesicular to bullous form;
- bullous necrotisation;
- deep necrotising non-bullous form; and
- allergic and toxic contact reactions of the skin.

Different forms of the above cutaneous lesions may be observed in one patient. The pigmentary exfoliative form is often combined with severe lung damage.[42]

Gastrointestinal tract
Gastrointestinal effects after SM exposure have been documented in some studies. Destruction of the mucosa and shedding of the epithelial elements, however, begin days after exposure, resulting in loss of large volumes of fluid and electrolytes.[40,43] Acute gastroduodenitis with haemorrhagic erosions, acute desquamative enteritis, and severe haemorrhagic necrotic colitis were reported in the WWI veterans[44] but not observed in the Iranian veterans.

Central nervous system
Extremely heavy exposure to SM can cause central nervous system (CNS) excitation leading to convulsions in animals.[15] Balali-Mood and Navaeian[19] reported convulsions in six Iranian veterans who were hospitalised during the early stages of their intoxication. Most casualties from the WWI and from the Iran–Iraq conflict, however, revealed mild and very non-specific neurological effects such as headache, anxiety, fear of the future, restlessness, confusion, and lethargy.[19]

Peripheral nervous system
A frequent long-term complication in patients exposed to SM is delayed neuropathic symptoms, which are under-represented in most previous studies.[45]

Haematopoietic system
As an alkylating agent, SM is particularly toxic to rapidly proliferating cells such as lymphoid and bone marrow cells. Leukocytosis is common within the first few days after exposure. White blood cell counts then begin to drop on the third and fourth days after exposure and reach their minimum level around the ninth day. This leukopaenia is followed by a decrease in megakaryocytes and finally in the erythropoietic series.[46–49] Bone marrow biopsies have shown hypocellular marrow and atrophy involving all elements.[14] If cytopaenia is not marked and there are still remaining stem cells, recovery will take place as the patient recovers.[15] The bone marrow studies reveal a severe decrease in cellularity and fat replacement, and nuclear changes, such as budding, double nuclear, and kariorrhexis in erythrocyte precursors. The toxic effects of SM on the haematopoietic system are dose dependent, and it is concluded that SM causes aplastic or ineffective haematopoiesis.[50] Severe leukopaenia, however, is an ominous sign, leading to secondary infections and higher mortality rates in these patients. SM victims with white blood cell counts of 200 cells/ml or less died during their initial admissions.[46] Aplastic anaemia in seven patients with SM poisoning six to 12 months after exposure was also reported.[2]

Immune system
SM poisoning can result in the impairment of both humoral and cellular immune functions.[46,49,51] Along with the appearance of clinical disorders, both C3 and C4 titres showed an increase, followed by a gradual decrease over one year. The majority of SM-exposed patients had increased levels of immunoglobulin (Ig)-G and IgM during the first weeks and up to the sixth month after exposure.[52]

Depression of cell-mediated immunity has been observed in the Iranian veterans one, two, and three years after exposure.[53] Natural killer cells, which are known to be one of the most important components of the cellular immunity, have been found to be significantly lower in patients with severe respiratory complications 10 years after exposure.[54] In a controlled study, the number of natural killer cells was still significantly lower 16–20 years after exposure.[6,49]

Endocrine system
SM may affect several different target organs comprising the endocrine system. The total and free testosterone levels were markedly decreased in the first 5 weeks after SM exposure, but all returned to normal by the twelfth week after exposure. A significant diminished sperm count in 77 SM veterans three to nine years after exposure have been reported.[34] Another study showed that 66% of 42 men examined one to three years after SM exposure had sperm counts of less than 3 million cells/ml and their follicle-stimulating hormone levels were higher than normal men.[2]

Clinical effects
Acute
The first contact with SM is usually painless and only a garlic or sulphur odour can be noticed. Normally, a symptom-free interval is observed for several hours. The duration of this interval correlates inversely with the absorbed dose of the agent. Eyes, skin, and the respiratory system are the three major targets for the local toxic effects of SM.

Initial clinical symptoms and signs occur in the eyes about one hour after exposure, starting with a sensation of grittiness, a progressive soreness, and a bloodshot appearance, proceeding to acute conjunctivitis. After several hours, the corneal epithelium begins to vesicate and slough, leading to severe pain, blepharospasm, and decreased visual acuity. Gradual spontaneous recovery usually occurs after 48 hours, with full regeneration of the corneal epithelium within four to five days. Complete symptomatic recovery, however, may take six weeks or longer.[5,12,13,16] Skin effects commence with erythema a few hours after exposure with no itching or pain leading to blister formation and further lesions within hours and days later as mentioned above.[52]

Respiratory symptoms usually occur earlier than skin lesion with dyspnoea, coughing, and chest discomfort progressing to acute rhinopharingo tracheobronchitis. In severe cases, bronchopneumonia, adult respiratory distress syndrome, and even pulmonary emboli may develop causing mortality mostly in the second week after exposure.[5,14,16]

The symptoms of systemic poisoning are very similar to those caused by radio- or chemotherapy. Low-dose exposure may result in headache, nausea, vomiting, and loss of appetite. Higher dose exposure may damage more severely the gastrointestinal tract and the bone marrow. This may

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result in immune suppression, leukopaenia, diarrhoea, fever, weakness, and, in very severe cases, excitation of the CNS with convulsions.[2,55] The maximum intensity of symptoms can be reached after days. An exposure to large doses of SM can cause damage to the haematopoietic and the immune system.[49,50]

Delayed

During the Iran–Iraq war, about 100,000 people suffered from SM exposure and now after 20 years, around 40,000 veterans have complained of delayed effects.[4,6,56] The first report of delayed toxic effects in Iranian veterans was reported in 1986 as mentioned above.[19] The most prominent late clinical effects were observed in the respiratory tract (78%), neuropsychiatrics systems (45%), skin (41%), and eyes (36%).[19,38]

Chronic

Chronic occupational exposure to SM is discussed above. The majority of clinical symptoms and signs were observed in the respiratory tract, presenting as coughing with or without productions, dyspnoea, wheezing, and bronchial rale. Chronic bronchitis, obstructive, and restrictive lung diseases leading to chronic obstructive pulmonary disease and bronchiectasis have been reported.[6] Malignancies, particularly in the respiratory tract and haematopoietic system, may occur.

Complications

It is very difficult to differentiate between the delayed toxic effects and complications of SM poisoning. However, complications may be defined as the persistent, permanent, and life-threatening delayed toxic effects of SM. Long-term complications of SM are discussed below.

Identification

Environmental

Environmental identification of SM in the field is of great medical importance to confirm the diagnosis, and to evaluate and decontaminate those exposed. Decontamination of the environment also requires agent identification.

Air

Identification of SM in the air is now possible using portable detectors, special biosensors that are available in some advanced chemical defence laboratories. It is also possible to quantify SM concentration in the air using a portable gas chromatograph–mass spectrometer.[57]

Soil

SM is a very stable compound and has been identified in the soil many months, and even years, after exposure under particularly cold environmental conditions. SM and its hydrolysis products including half-mustard and thiodiglycol can be identified and quantified by the specific and sensitive analytical methods such as gas chromatography–mass spectrometry (GC–MS).[58]

Water

SM is insoluble in water and its hydrolysis in the environment is very slow. However, identification of SM in water is possible in advanced environmental and CBRN defence laboratories by GC–MS.[57]

Patients

Alkylation products of SM with DNA and proteins (for example, haemoglobin and albumin), as well as its urinary metabolites, have been proven to be useful targets for the diagnosis of SM exposure in humans. Urinary markers are readily accessible, although their rapid elimination limits their use for retrospective detection. Adducts with macromolecules such as proteins offer longer lasting biological markers for exposure to SM, possibly up to several months.[59]

Blood

The primary site of DNA alkylation by SM is the N7 position of deoxyguanosine residues.[60] Upon depurination of the resulting N7-(2-hydroxyethylthioethyl)-0-2′-deoxyguanosine, N7-(2-hydroxyethylthioethyl) guanine (N7-HETE-Gua) is obtained. Although GC–MS analysis has proved problematic, N7-HETE-Gua can be conveniently analysed using liquid chromatography–mass spectrometry.[61] The adduct can be detected in urine, and also after processing of skin and blood samples of animals exposed to SM. An enzyme-linked immunosorbent assay (ELISA) has been successfully developed using monoclonal antibodies raised against N7-HETE-guanosine-5′-phosphate coupled to keyhole limpet haemocyanin.[62]

This method was applied to blood samples from two casualties of the Iran–Iraq War, collected 22 and 26 days following the alleged exposure to SM.[63] The alkylation of proteins by SM mainly occurs in carboxyl, amino, and sulphydryl groups, as well as in the nitrogens of the imidazole ring of histidine. Definitive evidence of specific alkylation sites can be obtained by using modern mass spectrometric (MS) techniques. Although MS methods can be used to confirm the diagnosis under more sophisticated conditions, the ELISA approach has been mainly developed for use under field conditions. Haemoglobin and albumin are two abundant proteins in human blood that can be readily isolated for the determination of SM adducts.[2,59]

Urine

Although the hydrolysis product of SM, namely thiodiglycol, is only a minor metabolite, the sulphoxide derivative of thiodiglycol is abundantly present in the urine and can be reduced to thiodiglycol for GC–MS analysis.[63]

Unfortunately, both thiodiglycol and its sulphoxide, are not unequivocal markers of poisoning in humans, and low concentrations are present in normal human urine.[64–67] The -lyase metabolites, which are derived from an initial reaction of SM with glutathione, are unequivocal biomarkers

and can be reduced to thioether derivatives for subsequent GC–MS analysis.[68] This method has been applied to urine samples from two human casualties accidentally exposed to the agent and from five Iranian casualties of CW attacks. The -lyase metabolites were detected in one sample collected 13 days after the alleged SM exposure.[68,69]

Blister fluid
Analysis of the vesicle fluid for thiodiglycol may confirm a suspected SM exposure. The fluid contained in the vesicles is not toxic and presents no risk to the attending medical staff.[70]

Tissue
The DNA adduct can also be detected in skin of human exposed as it was done after processing in animals exposed to SM.[62] Organ tissues of the post-mortem samples taken from chemical Iranian martyrs revealed SM with different concentrations in different organs.[31,32]

Post mortem
Toxicological analyses of the post-mortem samples (blood, urine, and organ tissues) of two Iranian chemical gassed combatants who died in Belgian hospitals, revealed SM.[32] The detailed biological fate of SM in Iranian chemical poisoned patients who were hospitalised in British hospitals including post-mortem toxicological analyses reported.[71]

Ethical considerations
All studies performed by the authors in this review followed the standard ethics. They were all confirmed by the University Medical Research Ethics Committee and were carried out after written informed consent of the patients was obtained. Figure 2 is from a patient whose consent for publication has been obtained.

Long-term complications
Information on the long-term effects of SM comes from two major lines of investigations: studies of soldiers who were exposed to the agent on the battlefield, and studies of workers who were employed in mustard gas factories (occupational exposure). Although long-term effects after battlefield exposure are referred to as 'late' or 'delayed' complications, the term 'chronic' is more suitable for the complications caused by occupational exposure. It must also be emphasised that delayed effects generally occur some months or years after a single or brief exposure and are not the same as chronic poisoning, which comes from continuous intake of the poison over a relatively long period of time. The first report on the delayed toxic effects of SM poisoning in 236 Iranian veterans revealed that the most common effects were on the respiratory tract (78%), CNS (45%), skin (41%), and the eyes (36%). These effects were recorded between two and 28 months after exposure.[21] Comparison of early (one week after exposure) and late (2 years after exposure) toxic effects of SM poisoning in 77 CWA
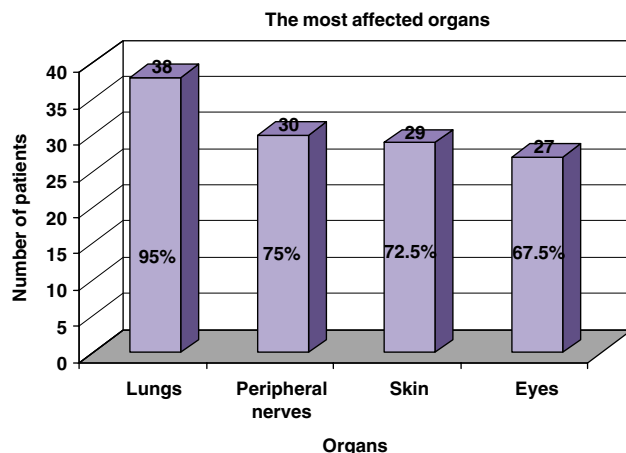

Figure 1 Frequency of delayed complications of SM in different organs of 40 Iranian veterans in Mashhad.

victims indicated that eye lesions do not change significantly, dermal complications tend to decrease, and respiratory complications generally deteriorate over the years.[72] In a study on 34,000 Iranians, 13–20 years after exposure to SM, the most common complications were found in the lungs (42.5%), the eyes (39%), and the skin (24.5%).[23] In a group of 40 severely intoxicated Iranian veterans in Mashhad, 16–20 years after their initial exposure, the most commonly affected organs were lungs (95%), peripheral nerves (77.5%), skin (75%), and eyes (65%) as shown in Figure 1.

Respiratory
Respiratory problems are the greatest cause of long-term disability among people with combat exposure to mustard gas. A triad of cough, expectoration, and dyspnoea has been found to be present in more than 80% of Iranian veterans three years after their initial exposure.[24] Haemoptysis (mainly streaky), chest tightness, chest pain, and nocturnal dyspnoea are also frequent. The main objective clinical findings are generalised wheezing (the most common sign), crackles, decreased lung sounds, clubbing, and cyanosis.[6,25]

Pulmonary function testing has revealed more obstructive patterns than restriction and about half of these obstructive spirometric results are reversible in response to inhaled bronchodilators. FVC, FEV1, and FEV1/FVC (FEV1%) have all been found to be significantly lower in SM-intoxicated veterans compared with healthy unexposed individuals and CWA survivors who had used a gas mask at the time of attack.[24] Abnormal spirometric findings, in general, and restrictive patterns, in particular, tend to increase over time.[2,19,73] A study on 77 subjects, who were present in a contaminated area and had no acute signs and symptoms at the time of exposure, but now have respiratory disorders, indicates that sub-clinical exposure to SM can be responsible for the occurrence of delayed respiratory complications such

as bronchiectasis and bronchiolitis obliterans.[7] Chest radiograph findings in patients with late respiratory complications of SM have been described as increased bronchovascular markings, hyperinflation, bronchiectasis, pneumonic infiltration, and radiological evidence of pulmonary hypertension.[19,74] However, chest radiographs are not sensitive enough for the detection of respiratory complications in these patients and high-resolution computed tomography of the chest may be required as the diagnostic imaging procedure of choice.[75] A study of 197 Iranian veterans 10 years after a single heavy exposure to SM revealed the development of a series of delayed destructive pulmonary sequelae such as chronic bronchitis (58%), asthma (10%), bronchiectasis (8%), large airway narrowing (9%), and pulmonary fibrosis (12%).[71]

Chronic bronchitis

Several studies have reported chronic bronchitis as the most common late complication of the respiratory system resulting from war exposure to mustard gas.[7,76,77] Hypoxaemia and hypercapnoea are commonly observed in moderate to severe cases, leading to cor pulmonale and respiratory failure in the final stages of the disease.[78,79] Infection of the respiratory tract, resulting in bronchopneumonia, is also a common problem, often complicated by septicaemia.[80]

Asthma

Airway hypersensitivity, manifested as typical attacks of breathlessness, wheezing, and nocturnal cough, as well as a reversible obstructive pattern on pulmonary function tests, have been reported between four weeks to 20 years after SM inhalation. Patients with chronic bronchitis may also have some degree of bronchospasm, which does not respond to bronchodilators. Attacks of bronchospasm are characteristically triggered by respiratory infections, environmental allergens, and cold weather.[77,78]

Bronchiectasis

Direct effects of SM on the bronchial wall mucosa and, more recurrent, respiratory infections following SM inhalations are known to be responsible for the development of bronchiectasis. Both the severity and frequency of bronchiectatic lesions tend to increase over the long-term follow-ups, as evidenced by a study of 40 Iranian veterans with severe late complications of SM poisoning. These lesions usually begin bilaterally in the lower lobes and then progress towards the middle lobe and the lingula. In severe cases with extensive bronchiectatic lesions, pulmonary hypertension, and ultimately cor pulmonale may occur.[7,78,81]

Large airway narrowing

Airway narrowing, due to scarring or granulation tissue, is a late sequel of acute injuries to the trachea and large bronchi, usually developing two years after exposure.[6,82,83] A study of 19 Iranian veterans with large airway narrowing due to SM, revealed stenosis in the trachea (seven patients), main

bronchi (eight patients), and lobar bronchi (four patients).[77] In contrast to stenosis caused by prolonged intubations, there is no predilection in the right main bronchus.[78,82] The major problem in these patients is the recurrence of the lesion, which usually occurs 6 months after treatment.[83]

Pulmonary fibrosis

Late-onset pulmonary fibrosis has been reported in several Iranian veterans with combat exposure to SM.[77,78] The analysis of bronchoalveolar lavage fluid from patients with mustard gas inhalation showed that these patients have an ongoing local inflammatory process of the lower respiratory tract resulting in the development of pulmonary fibrosis years after the initial exposure. Histopathological examination of transbronchial lung biopsies (TBLBs) of SM-exposed veterans revealed variegated fibrosis, diffuse fibrosis, and an absence of fibrosis in 86, 4, and 10% of the patients, respectively. Usual interstitial pneumonitis accounted for 97% of all cases of fibrosis.[84] In another study, electron microscopic examination of seven TBLB specimens was carried out in a WHO research centre in Japan. Abnormal findings included proliferation, desquamation, and degeneration of the bronchial epithelial cells; interstitial fibrosis or fibrosing alveolitis; and an increased type I and type II alveolar epithelial cells as well as hyperplasia of ciliated and goblet cells.[85] Inflammation and fibrotic processes in the lung tissue of SM-exposed patients may be progressive.[56] Diffusing capacity of the lung could be used as an objective monitor of the degree of fibrosis and also as a good predictor of prognosis.[77]

Dermal

The occurrence and persistence of lesions following SM exposure is directly related to the duration and severity of exposure. Injury that results in erythema and oedema without vesicle formation is almost always followed by a complete healing and no residual effects.[2,86] Blistering and necrotic wounds, however, cause permanent residual effects. The first report of delayed toxic effects of SM poisoning two years after exposure, in 236 Iranian veterans, revealed late skin effects such as hyperpigmentation (34%), hypopigmentation (16%), and dermal scarring (8%).[19] The most common skin complaint among these patients was itching followed by a burning sensation and desquamation. These symptoms are basically owing to dryness of the skin and thus become worse in dry weather and after physical activity. A more recent study of 40 Iranian veterans, who were heavily exposed to the gas 16–20 years previously, revealed the most common cutaneous lesions as hyperpigmentation, erythematous papular rash, dry skin, multiple cherry angiomas, atrophy, hypopigmentation, and hypertrophy. These lesions were found on the genital areas (48%), the back (48%), the front thorax and abdomen (44%), the lower extremities (mainly inguinal) (44%), the upper extremities (mainly auxiliary) (41%), and the head and neck (15%). Dry skin was more prominent in the extremities. Hyperpigmentation

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in some patients had the appearance of pigmented xerodermoid, which is a diffuse hyperpigmented area with superimposed macular hypo- and hyperpigmentations.[6,11]

In another study, the cutaneous lesions of 500 SM-exposed Iranian veterans were compared with that of 500 unexposed veterans. An association was found between SM exposure and late skin lesions such as severe dry skin, hyper- and hypopigmentation, local hair loss, eczema, and chronic urticaria. There was also a higher incidence of vitiligo, psoriasis, and discoid lupus erythematosus among SM-poisoned patients. This could be due to the immunological basis of these disorders and the fact that SM has adverse long-term effects on the immune system. Previously injured sites have been reported to be sensitive to subsequent mechanical injury and showed recurrent blistering after mild injury.[87]

Histopathological examination of skin biopsies has revealed non-specific findings including epidermal atrophy, keratosis, and basal membrane hyperpigmentation. Non-specific fibrosis and melanophages have also been observed within the dermis.[6,11,78,87] Areas (head and neck) of hyper- and hypopigmentations of a patient two years after SM exposure are shown in Figure 2. Occupational exposure to SM has been demonstrated to cause a variety of skin changes, including pigmentary disorders, skin ulcers, and cutaneous cancers.[88]

Ophthalmological
In less than 1% of patients with battlefield exposure to SM, a delayed type of ulcerative keratopathy may develop, leading to late-onset blindness.[89–92] The maximum delayed toxic effects usually occur 15–20 years after initial exposure, although latency periods as long as 40 years or as short as six years have also been reported.[13,93,94] Patients are usually symptom free for an indefinite number of years when delayed keratitis develops, characterised by photophobia, lacrimation, and failing vision.[92]

Figure 2 Hyperpigmentation of the blister sites in an Iranian veteran with delayed complication of SM.

In acute stages, the limbal region frequently presents a marbled appearance in which porcelain-like areas of ischaemia are surrounded by blood vessels of irregular diameter. Later, vascularised scars of the cornea are covered with crystal and cholesterol deposits, leading to a worsening of the opacification, recurrent ulcerations, and sometimes, corneal perforation. Opacification of the cornea is seen predominantly in the lower and central portions, whereas the upper part is often protected by the eyelid.[92,94] Surprisingly, lesions even recur after corneal transplantation.[93] The exact pathogenesis of this condition is unknown, but degenerative processes and immune reactions against corneal proteins (collagen–mustard compound) have been suggested as the cause of long-term damage.[94] Unfortunately, there has been no report on any long-term studies on mustard gas workers to determine their ocular status after prolonged occupational exposure.

Neuropsychiatric conditions
Casualties from WWI and from Iran–Iraq conflict were noted to have long-term mood and anxiety disorders, as well as post-traumatic stress disorder.[95,96] Debility, loss of vitality, impaired concentration, sensory hypersensitivity, diminished libido, weakened potency, neuralgic complaints, and disorders in autonomic regulation are the common manifestations. Neuropsychiatric evaluation of 1428 Iranian veterans, three to nine years after exposure to SM, revealed anxiety (15%), depression (46%), personality disorders (31%), convulsions (6%), and psychosis (3%).[38] Disorders of consciousness (27%), attention (54%), emotion (98%), behaviour (80%), thought process (14%), and memory (80%) were studied in 70 patients, three to five years after SM exposure.[97] Depression and post-traumatic stress in Iranian survivors of chemical warfare, mostly SM exposure, were also reported.[98] In another study, decreased libido and impotence were recorded in 52 and 9% of the patients, respectively. Interestingly, 10% of the patients revealed an increased libido. Functional photophobia, functional aphonia, and effort syndrome have also been reported.[6]

Neuromuscular conditions
Electromyography (EMG) and nerve conduction velocity (NCV), on 40 Iranian veterans with severe late manifestations of SM poisoning, revealed abnormalities in the peripheral nervous system of 77.5% of the patients. NCV disturbances were more common in sensory nerves compared with motor nerves and more prevalent in the lower extremities than in the upper extremities. EMG recordings revealed a normal pattern in 24 (60%) patients, incomplete interference with normal amplitude in six (15%) patients, and incomplete interference with low amplitude in 10 (25%) patients. NCV and EMG disturbances in both upper and lower extremities were mostly symmetric.[78]

Immunological and haematopoietic myelosuppression is the most serious effect of SM. SM can cause long-term effects on the immune system in patients with severe intoxication.

Table 1 Significant changes of haematological and immunological parameters in 40 patients with severe SM intoxication compared to 25 normal individuals

	Patient mean ± s.d.	Control mean ± s.d.	P-value
WBC (1000/mm^3)	7.24 ± 1.90	5.79 ± 1.12	0.025
RBC (million/mm^3)	5.46 ± 0.45	5.19 ± 0.28	0.035
Monocyte (%)	4.8 ± 1.6	3.9 ± 1.1	0.013
HCT (%)	48.3 ± 3.5	45.5 ± 1.9	0.047
IgM (mg/dl)	235.3 ± 84.8	136.8 ± 58.3	0.0001
C3 MIC (g/dl)	109.8 ± 30.1	90.9 ± 14.8	0.03
CD3 (%)	71.1 ± 8.6	65.6 ± 10.7	0.037
CD16+5 (NK cells)	11.6 ± 5.8	17.5 ± 9.6	0.006

Abbreviations: HCT, haematocrit; Ig, immunoglobulin; MIC, minimum inhibitory concentration; NK, natural killer; RBC, red blood cell; SM, sulphur mustard; WBC, white blood cell.

The impaired immunity is probably responsible for the increased risk of infections in these patients. Forty male subjects (aged 43.8 ± 9.8 years), who had confirmed SM poisoning 16–20 years before the study, were investigated. Significant changes of haematological and immunological parameters in these patients compared with 25 normal individuals are summarised in Table 1.[49]

Mutagenicity
A two-generation study of rats indicated that mustard gas was not teratogenic.[17,34] There has been no other report on the teratogenicity of SM and no clear evidence of this problem following human exposure, although there have been some claims made.

Carcinogenicity
SM is genotoxic because of its reactions with DNA, which is an important first step in carcinogenesis. Although most cells possess effective DNA repair mechanisms, these are not always effective in the case of SM damage. Alkylation of O_6-guanine by SM seems to be critical. O_6-ethylthioethyl-guanine is a poor substrate for the DNA repair enzyme O_6-alkylguanine-DNA alkyltransferase.[99] Therefore, this O_6-lesion may be the most important mutagenic lesion. However, only limited data are available on the specific mutations produced by SM. Mutations in a tumour suppressor or an oncogene gene can favour a proliferate advantage of a clonal cell. Notably, alterations in the p53 tumour suppressor gene have been described in Japanese mustard gas workers.[100]

However, most of the lesions in this population were similar to smoking-related mutations. Mutations in lymphocytes at the hypoxanthine phosphoribosyltransferase (*hprt*) gene locus have also been reported.[101]

Reproductive
The effects of SM exposure during pregnancy are unknown. Data addressing the productive toxicities of SM in human models are both lacking and contradictory.[102]

Management
Specific
No specific antidote has been developed for the treatment of SM. Sodium thiosulphate and *N*-acetylcysteine have been considered, although the acute clinical efficacy of these agents has yet to be established. Sodium thiosulphate infusion (10%) may prevent the toxic manifestations of SM, providing it is administered immediately after an exposure and no later than 30 min after exposure.[103,104] There is no specific treatment for the delayed toxic effects and complications of SM in different target organs.

Supportive
Supportive care focuses on the prevention of infection and reduction of pain. Given the range of chronic health effects of SM, patients are best managed by multidisciplinary clinical teams of specialists. Financial, social, and cultural support together with health education to maintain a good lifestyle is of great importance.[2]

Conclusions and recommendations
The use of SM as an incapacitating warfare agent in the past century, particularly in WWI and the Iraq–Iran conflict, has proved its highly long-lasting toxic effects. This experience may ensure further use of the agent in future military and terrorist attacks. SM exerts its toxicity through a number of postulated pathogenic mechanisms including DNA alkylation, NAD depletion, and inactivation of glutathione. The eyes, skin, and respiratory system are the three major targets for the direct toxic effects of SM. When absorbed in large amounts, it can also damage rapidly proliferating cells of the bone marrow and cause severe suppression of the immune system, as well as other systemic toxicities such as neurological and digestive disorders. Even more important is a wide range of chronic health effects including chronic bronchitis, bronchiectasis, frequent bronchopneumonia, and pulmonary fibrosis, all of which tend to deteriorate in time. Severe dry skin, delayed keratitis, and pathogenic status of cell-mediated immunity with a subsequent increased risk of infections and even possible malignancies are also among the most distressing long-term consequences of SM intoxication. However, there are still major gaps in SM literature and further studies on humans exposed to the agent are required. Immunological and psychological dysfunctions, as well as the relationship between SM exposure, and carcinogenesis and teratogenesis are important fields that require further investigation. There is also a paucity of information regarding the medical management of acute and delayed toxic effects of SM poisoning, a subject that greatly challenges health care specialists.

It is hoped that by the advancement of the Organization for Prohibition of Chemical Weapons (OPCW), no CWA will be used in the future. However, chemical terrorism involving the use of SM remains a threat to human health globally. Thus, further studies on this subject are recommended.

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