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Review Article

THE CLINICAL TOXICOLOGY OF SULFUR MUSTARD

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Sulfur mustard (SM) or mustard gas is one of the most important agents of chemical warfare due to its simple and cheap chemical synthesis that makes it readily available for both military and terrorist use. SM acts as an alkylating agent that induces disruption of nucleic acids and proteins, impairing cell homeostasis and eventually causing cell death. It rapidly reacts with ocular, respiratory, and cutaneous tissues, as well as bone marrow and the mucosal cells of the gastrointestinal tract, resulting in several devastating long-term effects on human health, many of which are not clinically nor pathologically well defined. In light of the possible threat of SM use against military and civilian populations, physicians should be aware of its grave effects and know how to care for its victims. The pattern of immediate and long-term toxic effects following exposure to SM is reviewed with special references to the recent data on clinical and paraclinical investigations and management of more than 100,000 chemical war casualties incurred during the Iran-Iraq conflict.

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Introduction

Among the available chemical warfare agents (CWA), sulfur mustard (SM), also known as mustard gas, has been the most widely used chemical weapon. It was the most effective chemical warfare agent used during World War I (WWI) and this efficacy earned for mustard gas the sobriquet "King of the Battle Gases".[1, 2] Although there are presently more toxic chemical warfare agents, mustard gas has remained the chemical weapon of choice in modern tactical warfare, as evidenced by its use during the Iran-Iraq conflict between 1983 and 1988.[3]

In the USA, stock piles of this agent were scheduled for destruction, which obviously required handling cares to prevent environmental and occupational exposure.[4] Health professionals, particularly physicians, who care for patients in an acute care setting, as well as those responsible for managing chronic and late complications of such toxic agents, must be familiar with the management of patients exposed to SM. The aim of this review is thus to provide an overview of the short- and long-term toxic effects of exposure to SM as well as the basic principles of medical management in this area. The data presented here have been obtained from both a comprehensive review of the literature on SM and also from our own first-hand experience in managing thousands of patients who were referred to our university teaching hospital after SM exposure and during their long-term follow-ups.

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History

The first use of SM as chemical warfare was on July 12, 1917, in a field near Ypres, Belgium, where during 10 days of attack more than one million mustard shells were fired at Allied troops by Germans. Thereafter, it was responsible for more than 80% of all documented chemical casualties.

After WWI, a widespread campaign to ban chemical warfare was mounted and the Geneva Protocol was promulgated in 1925. However, the Italian campaign in Ethiopia (1935 – 1936), Italian troops used mustard gas on a large scale against

unprotected native forces. Despite the production of large quantities of chemical warfare munitions, SM was not used in combat during the Second World War (WWII). In December 1943, however, an Allied ship carrying large quantities of mustard gas exploded in the harbor of Bari, Italy, dispersing the agent and causing more than 600 casualties.[5]

Accusations and reports regarding the use of chemical weapons have been common after WWII. The use of SM by Egyptian forces in Yemen (1963 – 1967) seems to be better supported than most.[6] The greatest use of SM recently, however, has been by the Iraqi army against Iranian soldiers and even against its own Kurdish population between 1983 and 1988.[3] In one particularly distressing event, some 5,000 Kurdish civilians were killed in the Iranian-occupied village of Halabja in 1988. Several CWAs, including SM and sarin, were identified in this massacre.[7]

Pharmacology and toxicology of sulfur mustard

There are two principal groups of mustards. Sulfur mustard bis (2-chloroethyl) sulfide, which is the chemical vesicant used as a warfare agent, and nitrogen mustard tris-(chloroethyl) amine, which was developed in WWII, but found to be unsuitable for munitions. Nitrogen mustard is closely related chemically and toxicologically to SM and is currently a useful chemotherapeutic agent known as Mustargen.[8]

Physicochemical properties of sulfur mustard

SM [$S(CH_2CH_2Cl)_2$] is a poorly volatile oily liquid, which is barely soluble in water (0.07% at 10ºC) and highly soluble in organic solvents, fuels, and lubricants. It ranges from light yellow to dark brown in color and has the odor of onion, garlic, or mustard, hence its name. Low volatility along with low solubility in water leads to lengthy persistence of the compound in the field. Terrain and all objects present including foodstuffs, porous materials, paint, and varnish coatings will become contaminated by SM for very long periods of time, particularly in cold and damp climates. The formula and some of the physicochemical characteristics of SM are shown in Table 1.[8, 9]

Absorption of sulfur mustard

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 vapor, skin plays a very important role as a port of entry for SM. Renshaw (1946) noted that SM liquid or saturated vapor penetrates human skin at a rate of 1 to 4 $mg.cm^2$/min at 21ºC.[10] Any increase in ambient temperature causes an increased penetration. Most (80%) of the liquid form of SM evaporates; of the 20% that penetrates, 12% is retained in the skin and about 8% is absorbed systemically. Therefore, large dosages of SM vapor delivered at 1,000 – 10,000 $mg.min/m^3$, or liquid at 40 – 100 mg/cm^2, over a long exposure time will yield significant systemic toxicities.[10, 11] Cameron et al (1946) demonstrated that inhaled SM, in rabbits, is absorbed more readily and about 80% of the SM vapor is absorbed from the upper airways.[12]

Table 1. Chemical identity and physicochemical constants of sulfur mustard.

Chemical formula	$C_2 H_4 Cl_2 S$
Chemical synonyms	1, 1'-thiobis (2-chloroethane), bis-(2-chloroethyl) sulfide, 2, 3'-dichloroethyl sulfide
Melting point	13 – 14˚C
Boiling point	215 – 217˚C
Vapor pressure	0.72 mmHg at 20˚C
Liquid density	1.274 (g/cm^3)
Molecular weight	159.08 D

Mechanism of action of sulfur mustard

The 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 has been made that it is DNA alkylation, which plays an important role in delayed healing.[13 – 15] The major alkylating site of nucleic acids in mammalian origin is the nitrogen residue of guanine (Figure 1).[16]

Cell death from DNA cross-linking is delayed until the cell replicates its DNA or undergoes division. At higher cellular exposure, 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 experienced with SM 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 sulfhydryl-containing proteins and peptides, such as glutathione. These sulfhydryl compounds are critical in maintaining the appropriate oxidation-

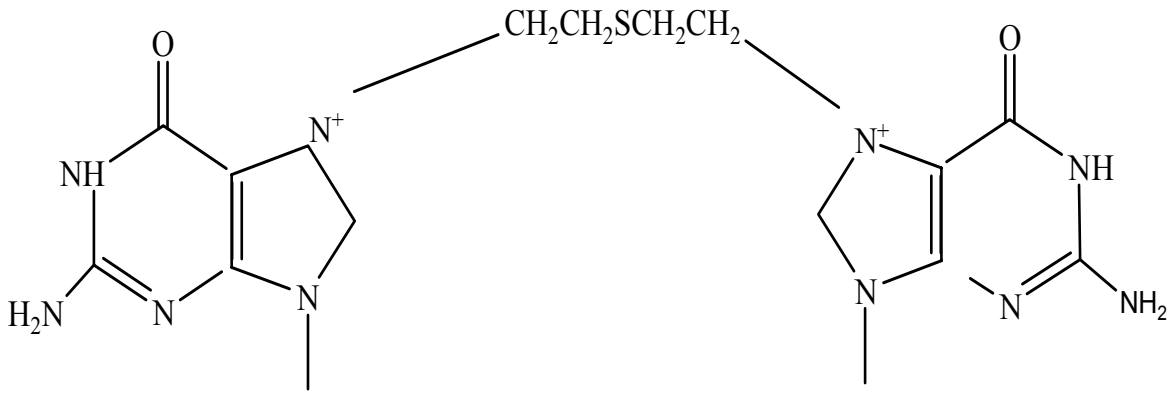

Figure 1. Cross-linking of guanine residues by sulfur mustard.

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.[17, 18]

General toxicity

The toxicity of SM as an incapacitating agent is of much greater importance than its capacity to kill in terms of lethal dose 50% (LD_{50}). In fact, compared with the nerve agents, SM has a relatively low acute lethal toxicity.[19] Among the survivors of mustard gas attacks in WWI and in the Iran-Iraq War, nearly all victims suffered from skin and eye burns and respiratory injuries.[20] However, reported fatality rates were low, with less than 2% of the exposed soldiers during WWI and 3 – 4% in the Iran-Iraq conflict.[21]

Intravenous LD_{50} figures in rats and mice are 3.3 $mg.kg^{-1}$ and 8.6 $mg.kg^{-1}$, respectively.[20] The LD_{50} for humans is between 200 mg (when swallowed) and 4 – 5 g (when applied to the bare skin over a long exposure time). The respiratory lethal dosage is estimated at 1,500 $mg.min/m^{-3}$ of $C_{(t)}$ (the product of concentration [$mg.m^{-3}$] and exposure duration [min]).[22] Effects of exposure to different concentration-time products of SM are listed in Table 2. [23 – 28]

Acute toxic effects

Acute toxic effects generally appear after variable periods of latency (Table 3), [24 – 28] depending on the dose, mode of exposure, the environmental temperature, and probably on the individual.

Table 2. Effects of exposure to different concentration-time products of sulfur mustard.

Exposure dose ($mg.min/m^3$)	Effects
50	Onset of eye effects
100 – 400	Onset of respiratory and skin effects
200	Total incapacitation from the eye and respiratory effects
200 – 1000	Onset of skin burns
750 – 10000	Severe incapacitation from skin burns

Eyes

Eyes are the most sensitive organs to SM.[29, 30] 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. The first clinical signs occur about one hour after exposure, starting with a sensation of grittiness, progressive soreness, and a bloodshot appearance, then proceeding to edema and all the phenomena of acute conjunctivitis. At 2 – 6 hours postexposure, patients complain of severe ocular pain, lacrimation, photophobia, and sometimes even temporary blindness. Physical findings include blepharospasm, periorbital edema, conjunctival injection, and inflammation of the anterior chamber.[25, 29, 31 – 33]

While concentrations of less than 50 to 100 $mg.min/m^{-3}$ cause simple conjunctivitis, corneal swelling and edema occur with doses exceeding 200 $mg.min/m^{-3}$. After several hours, the corneal epithelium begins to vesicate and slough, leading to a decreased visual acuity. At even higher doses, corneal ulceration may occur, with significant visual impairment and the risk of permanent blindness.[34,35] Khakshoor (1988) described immediate corneal injuries in SM-injured patients as superficial punctate keratitis, corneal abrasion, superficial infiltration, whorl pattern dystrophy, and corneal ulcer. No evidence of cataract, glaucoma, and retinal injuries has been found in the acute stage.[36] Gradual spontaneous recovery usually occurs after 48 hours of severe pain and blepharospasm, with full regeneration of

Table 3. Latency periods from exposure to the onset of signs and symptoms in sulfur mustard-intoxicated patients.

Time postexposure	Symptoms and signs
- Minute	Nausea, vomiting, and smarting of the eyes
- Hours	Intense burning eye pain, lacrimation, and photophobia from blepharospasm; rhinorrhea, sneezing, and sore
- Hours	Erythema develops on skin, followed by blisters; aphonia, hoarseness, and nonproductive cough
- Hours	Blistering becomes more marked, there is intense itching of skin; productive cough develops and sloughed tissue is expectorated; eye effects are maximal at this time
- Day	Gradual ocular recovery starts; hyperpigmentation occurs on and around the burned area; secondary infections may supervene leading to bronchopneumonia
Day - week	Complete symptomatic recovery from eye problems, although roughening of the cornea may be observed; blisters heal slowly; Respiratory problems resolve slowly, although some cough and huskiness may persist for as long as weeks

the corneal epithelium within 4 – 5 days. Complete symptomatic recovery may take 6 weeks or longer.[37]

Respiratory tract

Next to the eye lesions, the greatest discomfort produced by mustard gas results from irritation and injury of the respiratory system.[29, 38] Respiratory effects occur in a dose-dependent manner from the nasal mucosa to the terminal bronchioles. Initial, or perhaps the only effect, is pain and discomfort in the nose or sinuses with increased nasal secretions, sneezing, and sore throat, usually developing 4 – 16 hours after exposure. Rhinorrhea is often profuse and epistaxis may occur. Larger amounts of vapor will cause laryngeal injury (aphonia or husky voice) and damage to upper medium-sized airways (tracheobronchitis), which is usually manifested by a nonproductive hacking cough. [26, 28, 39]

After a very large amount of vapor, there will be damage to the terminal airways with productive cough, dyspnea, and possibly hemorrhage into the alveoli.[40] Coughing may be severe and sputum is often purulent.[20, 41] Necrosis of the mucosa with associated inflammation can lead to the formation of a diphtheritic-like membrane in most severe cases. This can occur at any level and may obstruct the airway or break off to obstruct lower airways.[42] Later, the clinical picture of adult respiratory distress syndrome may be present as seen in the Iranian victims who suffered multisystem organ failure.[20, 43]

Infection of the respiratory tract, resulting in bronchopneumonia, is a common complication, usually developing 36 – 48 hours after exposure. It may terminate in bronchopneumonia, with death at any time between the second day and the fourth week.[24, 40, 44, 45] Although recovery can be rapid, some irritation, cough, and huskiness may persist for as long as 6 weeks. Prolonged recovery (1 – 2 months) can be expected, particularly after secondary infections and necrotic bronchopneumonia. Experience with Iranian casualties showed that, in those with severe lung complications requiring artificial ventilation, prognosis was very poor, even when sophisticated facilities and intensive care therapy were applied.[40, 41]

Skin

The characteristic skin lesion of SM is erythema followed by blisters.[40, 46, 47] Erythema usually begins 2 to 24 hours after vapor contact and is accompanied by extreme itching, which diminishes as blisters appear. [40, 41] Typical blisters initially appear as small vesicles within the area of erythema about 18 hours after contamination. They gradually coalesce to form the characteristic pendulous blisters, containing large volumes of a clear yellow fluid. Blisters are not painful *per se* but they may be uncomfortable and feel tense. Bullous lesions are particularly likely to occur on warm, moist areas such as genitalia, axilla, and areas where tight clothing is worn.[47, 48]

At 48 hours postexposure, blistering becomes more marked and fresh corps of blisters appear. Large blisters usually break, leading to erosions and full-thickness skin loss and ulceration. Necrosis may occur at these sites, followed by the formation of an eschar at 72 hours postexposure. The eschar usually begins to slough by 4 – 6 days, leaving a pigmented scar by 19 days.[49, 50] Increased darkening from an increased melanogenesis is

characteristically seen in the affected skin, as well as in the periphery of mustard-induced blisters.[5, 40]

Helm and Balali-Mood (1988) classified the cutaneous mustard gas lesions as follows:

- erythematous form;
- pigmentary exfoliation;
- superficial vesicular to bullous form;
- bullous necrotization;
- deep necrotizing nonbullous 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.[51]

The burn caused by a blister agent like SM is much slower to heal in comparison to a thermal burn. To some extent, the delay in healing of SM burns is dependent on the burn area. For larger burns the delay in healing may be much slower than for thermal burns of comparable area.[49] In mild cases, skin lesions may remain limited to an erythema, which turn black in about 10 – 15 days, while the superficial epidermal layers desquamate without causing any actual skin defect. This phenomenon, already known from WWI [52, 53] and II,[5] was also observed in the Iranian casualties.[40] With moderate to severe exposure, large blisters develop. Blisters usually heal in 2 or 3 weeks, and full-thickness erosions after 6 or 12 weeks. Characteristically, the healed area loses its pigments; whilst the area of sublethaly damaged cells surrounding the original lesion becomes hyperpigmented (Figure 2). The site of healed mustard burns is hypersensitive to mechanical trauma.[49, 54]

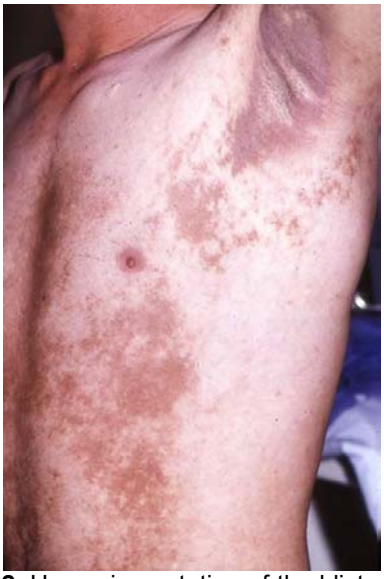

Figure 2. Hyperpigmentation of the blister sites 8 weeks after SM exposure in an Iranian veteran.

Bone marrow

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 (WBC) counts then begin to drop on the third and fourth days after exposure and reach their minimum level around the ninth day. This leukopenia is followed by a decrease in megakaryocytes and finally in the erythropoietic series.[40, 55, 56] Bone marrow biopsies have shown hypocellular marrow and atrophy involving all elements.[43] If cytopenia is not marked and there are still remaining stem cells, recovery will take place as the patient recovers.[57]

The bone marrow studies by Tabarestani and co-workers (1988) revealed a severe decrease in cellularity and fat replacement, nuclear changes such as budding, double nuclear, and kariorrhexis in erythrocyte precursors. They showed that the toxic effects of SM on hematopoietic system are dose dependent and concluded that SM causes aplastic or ineffective hematopoiesis.[58]

Severe leukopenia, however, is an ominous sign, leading to secondary infections and higher mortality rates in these patients. Willems (1989) stated that all SM victims with WBC counts of 200 cells/mL or less died during their initial admissions.[40] Aplastic anemia in seven patients with SM poisoning 6 – 12 months after exposure was also reported.[59]

Other clinical effects

Gastrointestinal (GI) effects following SM exposure have been documented in some studies. The most common gastrointestinal symptoms in the Iranian victims have been reported as nausea, vomiting, anorexia, abdominal pain, and diarrhea.[20] Transient nausea and vomiting in the first 24 hours of exposure is thought to be a reflex action and does not implicate damage to the GI mucosa.[29] 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.[24, 60] Canelli (1918) diagnosed gastrointestinal findings as acute gastroduodenitis with hemorrhagic erosions, acute desquamative enteritis, and severe hemorrhagic

necrotic colitis and called attention to the selective action of mustard gas on the GI tract.[61]

Extremely heavy exposure to SM can cause central nervous system (CNS) excitation leading to convulsions in animals.[57] Balali-Mood and Navaeian (1986) reported convulsions in six Iranian veterans who were hospitalized during the early stages of their intoxication.[20] Most casualties from WWI and from the Iran-Iraq conflict, however, revealed mild and very nonspecific neurologic effects such as headache, anxiety, fear of the future, restlessness, confusion, and lethargy.

Delayed toxic effects

Evidence on long-term effects of SM comes from two major lines of investigations: (1) studies of soldiers who were exposed to the agent on the battlefield and (2) studies of workers who were employed in mustard gas factories (occupational exposure). While long-term effects following battlefield exposure are referred to as "late" or "delayed" complications, the term "chronic" complication seems to be more suitable for the injuries caused by occupational exposure. It must also be emphasized 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 2 and 28 months after exposure.[62] Comparison of early (one week after exposure) and late (two years after exposure) toxic effects of SM poisoning in 77 CWA victims indicated that eye lesions do not change significantly, and dermal complications tend to decrease, and respiratory complications generally deteriorate over the years (Figure 3).[63] In a study by Khateri et al (2002) on 34,000 Iranians, 13 – 20 years after exposure to SM, the most common complications were found in the lungs (42.5%), eyes (39%), and the skin (24.5%).[64] More recently Balali-Mood et al (2004) described the toxic effects of SM poisoning in a group of 40 severely intoxicated Iranian veterans, 16 – 20 years after their initial exposure. The most commonly affected organs in this study were lungs (95%), peripheral nerves (77.5%), skin (75%), and the eyes (65%).[65]

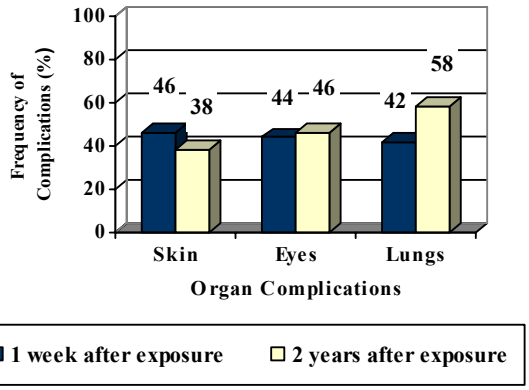

Figure 3. Comparison of early and late toxic effects of SM poisoning in 77 Iranian veterans.

Respiratory system

A. Battlefield exposure

Respiratory problems are the greatest cause of long-term disability among people with combat exposure to mustard gas. A triad of cough, expectoration, and dyspnea has been found to be present in more than 80% of Iranian veterans three years after their initial exposure.[66] Hemoptysis (mainly streaky), chest tightness, chest pain, and nocturnal dyspnea are also frequent. The main objective clinical findings are generalized wheezing (the most common sign), crackles, decreased lung sounds, clubbing, and cyanosis.[44, 62, 67]

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 in comparison to healthy nonexposed subjects as well as to those CWA survivors who had used a gas mask at the time of attack.[66] Abnormal spirometric findings in general and restrictive patterns in particular tend to increase over time (Figure 4).[65] A study by Ghanei and colleagues (2003) 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 subclinical exposure to SM can be responsible for the occurrence of delayed respiratory complications such as bronchiectasis and bronchiolitis obliterans.[68]

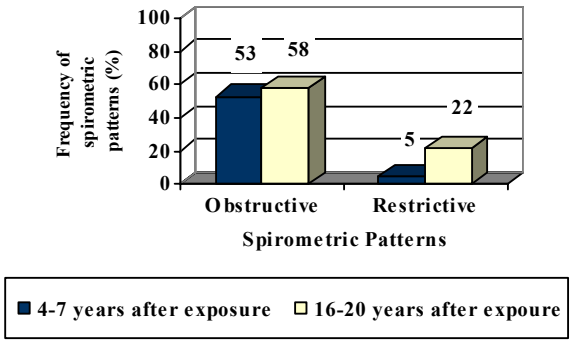

Figure 4. Comparison of obstructive and restrictive spirometric patterns, 4 – 7 years and 16 – 20 years after SM exposure in 40 Iranian veterans.

Chest X-ray (CXR) findings in patients with late respiratory complications of SM have been described as increased bronchovascular markings, hyperinflation, bronchiectasis, pneumonic infiltration, and radiologic evidence of pulmonary hypertension.[65, 69] However, CXR is not sensitive enough for detection of respiratory complications in these patients and high resolution computed tomography (HRCT) of the chest may be required as the diagnostic imaging procedure of choice.[70]

A study of 197 Iranian veterans 10 years after a single heavy exposure to SM revealed that there could be 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] Each of these complications is described in more detail below.

1- Chronic bronchitis: Several studies have reported chronic bronchitis as the most common late complication of the respiratory system resulting from combat exposure to mustard gas.[65, 69, 71 – 73] Hypoxemia and hypercapnea are commonly observed in moderate to severe cases, leading to cor pulmonale and respiratory failure in the final stages of the disease.[65, 66] Infection of the respiratory tract, resulting in bronchopneumonia, is also a common problem, often complicated by septicemia.[73]

2- 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 twenty 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.[65, 69, 71, 74]

3- Bronchiectasis: Direct effects of SM on bronchial wall mucosa and more importantly recurrent respiratory infections following mustard gas inhalation are known to be responsible for the development of bronchiectasis. Both the severity and frequency of bronchiectatic lesions tend to increase over 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 toward the middle lobe and the lingula. In severe cases with extensive bronchiectatic lesions, pulmonary hypertension and ultimately cor pulmonale may occur.[65, 70, 75, 76]

4- 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.[42, 65, 71, 77, 78] A study of 19 Iranian veterans with large airway narrowing due to SM, revealed stenosis in the trachea (7 cases), main bronchi (8 cases), and lobar bronchi (4 cases).[71] In contrast to stenosis caused by prolonged intubations, there is no predilection in the right main bronchus.[65, 77] The major problem in these patients is the recurrence of the lesion, which usually occurs six months after treatment.[78]

5- Pulmonary fibrosis: Late onset pulmonary fibrosis has been reported in several Iranian veterans with combat exposure to SM.[65, 69 – 71, 79] The analysis of bronchoalveolar lavage fluid from patients with mustard gas inhalation shows 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.[80]

Histopathological examination of transbronchial lung biopsies (TBLB) of 73 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 (UIP) accounted for 97% of all cases of fibrosis.[81] In another study, electron

microscopic examination of seven TBLB specimens was carried out in a WHO research center in Japan. Abnormal findings included: (1) proliferation, desquamation, and degeneration of the bronchial epithelial cells; (2) interstitial fibrosis or fibrosing alveolitis; and (3) an increased type I and type II alveolar epithelial cells as well as hyperplasia of ciliated and goblet cells.[82]

Aghanouri et al (2004) indicated that inflammation and fibrotic processes in the lung tissue of SM-exposed patients may be progressive.[79] Diffusing capacity of the lung (D_{LCO}) could be used as an objective monitor of the degree of fibrosis and also as a good predictor of prognosis.[71]

B. Occupational exposure

Several studies suggest that workers who were chemically exposed to mustard agents in British and Japanese munition factories developed chronic respiratory effects. In a cohort mortality study of 3,500 workers at a manufacturing plant in England, a statistically significant excess in the amount of death due to influenza, pneumonia, bronchitis, and asthma was found. This was present even among those with less than three years of employment at the plant and so was not related to the duration of exposure.[83]

A twenty-five year follow-up study of workers exposed to SM in a Japanese production plant revealed that highly exposed workers had more chronic bronchitis and a slightly lower FEV1/FVC ratio than either the less-exposed or unexposed group of their co-workers.[84] In another study, Brown (1949) reported that a large number of employees working at the Huntsville Arsenal in Alabama, who were continuously exposed to the gas over long periods of time, developed bronchiectasis with progressive emphysema and narrow attenuated bronchioles.[85]

Cancer of the respiratory system has also been associated with occupational exposure to SM and will be discussed later in this article (carcinogenicity).

Skin

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 edema without vesicle formation is almost always followed by a complete healing and no residual effects.[54, 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%), hypo-pigmentation (16%), and dermal scar (8%).[20] The most common skin complaint among these patients was itching followed by a burning sensation and desquamation. These symptoms are basically due to dryness of the skin and thus become worse in dry weather and after physical activity.

A more recent study on 40 Iranian veterans, who were heavily exposed to the gas 16 to 20 years ago, revealed the most common cutaneous lesions in the order of 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%), lower extremities (mainly inguinal) (44%), upper extremities (mainly axillary) (41%), and the head and neck (15%). Dry skin was more prominent in the extremities. Hyperpigmentation in some patients had the appearance of pigmented xerodermoid, which is a diffuse hyperpigmented area with superimposed macular hypo- and hyperpigmentations.[65]

In another study, Fekri and Janghorbani (1992) compared cutaneous lesions of 500 SM-exposed Iranian veterans with those 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 can 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 nonspecific findings including epidermal atrophy, keratosis, and basal membrane hyperpigmentation. Nonspecific fibrosis and melanophages have also been observed within the dermis.[65, 87]

Occupational exposure to SM has been demonstrated to cause a variety of skin changes, including pigmentary disorders, skin ulcers, and cutaneous cancers.[88] A study of 488 former

workers in a gas factory in Japan revealed 155 cases with pigmentation abnormalities consisting of hyperpigmented and depigmented raindrop spots, even on covered skin of the trunk and extremities. Another 22 cases with Bowen's disease, basal cell carcinoma, and hyperkeratotic papular eruptions were also described.[89, 90]

Eyes

In less than 1% of patients with battlefield exposure to SM, a delayed type of ulcerative keratopathy may develop, leading to late-onset blindness.[91 – 94] The maximum incidence usually occurs 15 to 20 years after initial exposure, although latency periods as long as 40 years or as short as 6 years have also been reported.[95, 96] Patients are usually symptom-free for an indefinite number of years, when delayed keratitis develops, characterized by photophobia, lacrimation, and failing vision (Figure 5).[94]

In acute stages, the limbal region frequently presents a marbled appearance in which porcelain-like areas of ischemia are surrounded by blood vessels of irregular diameter. Later, vascularized scars of the cornea are covered with crystal and cholesterol deposits, leading to worsening of 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.[34, 94, 96] Surprisingly, lesions even recur after corneal transplantation.[95] 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.[96]

Unfortunately, there have been no long-term studies on mustard gas workers to determine their ocular status after a prolonged occupational exposure.

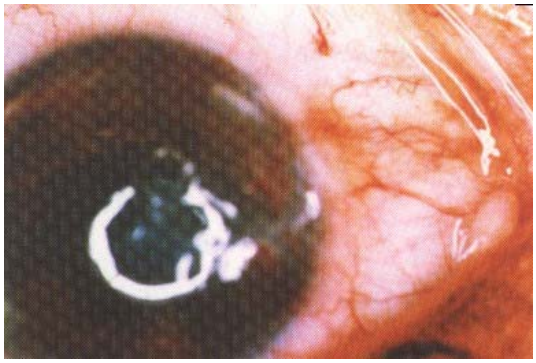

Figure 5. Delayed corneal vascularization and opacification as a result of sulfur mustard exposure.

Immune system

Available evidence suggests that exposure to SM could result in the impairment of both humoral and cellular immune functions.[40, 97] Dayhimi et al (1988) studied 100 CWA victims through one year after their exposure. None of the immunoglobulin classes showed out of range fluctuations, although an early increase and a slow return in IgG and IgA levels were noticed. Along with the appearance of clinical disorders, both C3 and C4 titers showed an increase, followed by a gradual decrease over one year.[98] The percentage of patients with increased levels of C3 and C4 remained higher 3 years after exposure especially in the severely poisoned patients.[97] Razavi et al (1988) studied CH50 in 120 Iranian combatants, in a period of maximum 51 days after exposure. The mean level of CH50 increased in the first two weeks, but began to decrease after the second week, reaching a level not significantly different from controls. Complement changes are probably related to the acute phase response following possible infections and, anyhow, indicate the efficiency of the classic pathway of the complement system.[99]

Hassan and Ebtekar (2002) demonstrated that the majority of SM-exposed patients had increased levels of IgG and IgM during the first weeks and up to the 6th month after exposure. Even eight years after contact, the percentage of patients with increased IgM, IgG, and IgE were still significantly higher than the controls.[100] A study of 40 Iranian veterans with severe late manifestations of SM poisoning indicated that the IgM level was still significantly higher 16 – 20 years after exposure, compared with the control group.[65]

Depression of cell-mediated immunity has been observed in the Iranian veterans one, two, and three years after exposure.[101] Natural killer (NK) 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.[102] Balali-Mood et al (2004) indicated that 16 – 20 years after exposure the number of NK cells were still significantly lower in patients than in the controls. No correlation was found between NK cell decrement and the severity of clinical complications in any of the target organs including the respiratory tract, skin, or the eyes.[65]

Occupational exposure to SM has been reported to cause impairment of NK cells in Japanese poison gas workers[103] and is known to be the major cause of recurrent infections, septicemia, and a higher incidence of malignancies in these patients.[65, 102]

Carcinogenicity

Based on laboratory studies, SM is classified as a carcinogen. Human studies indicated a causal association between occupational exposure to SM and the excessive occurrence of respiratory cancer, skin cancer, and possibly leukemia.[104] A significant excess of death (33 cases against 0.9 expected) due to respiratory cancer was found among former workers of the Japanese poison gas factory from 1929 to 1945.[105] Similarly, highly significant excesses in cancer of the larynx, pharynx, and other upper respiratory sites were observed in former employees of a British plant, manufacturing SM. A moderate, but still very significant excess mortality was also observed in lung cancer.[106] Gastric cancer, basal cell carcinoma, Bowen's disease, Bowen's carcinoma, and skin spinocellulare have all been reported following occupational exposure to mustard gas.[74, 83, 90]

Although an excessive amount of lung cancer is suggestive of an association, the battlefield experience is still somewhat more equivocal. Observations on American veterans of WWI suggest that the incidence of lung cancer was slightly more in men who had been exposed to mustard gas.[107] British soldiers, who received a pension for mustard gas poisoning, were found to have a raised mortality rate from lung cancer. However, most of these men also had chronic bronchitis and a similar excess of lung cancer was found in patients with bronchitis who had not been exposed to the gas.[108]

Carcinoma of the nasopharynx, bronchogenic carcinoma, adenocarcinoma of the stomach, as well as acute myeloblastic and lymphoblastic leukemia, have been reported in Iranian veterans.[74, 109, 110] Quantitative risk assessment, however, cannot be developed from the available data and a long-term follow-up is required to discover the exact relationship between battlefield exposure to SM and carcinogenicity.

Reproductive system

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.[60] Nevertheless, the damage is usually transient, because testicular recovery is observed at 2 weeks, with the formation of mature sperms 4 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/d 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.[111] McNamara et al (1975) also found no evidence of teratogenesis in rats treated with 0.5 to 2.0 mg/kg SM via gastric intubation from day 6 to 15 of gestation. Because fetal defects were observed only at the dose level that caused maternal toxicity, the investigators suggested that mustard gas was not teratogenic in rats.[25]

Data addressing the reproductive toxicities of SM in human models are both lacking and contradictory. Azizi et al (1995) described acute and chronic effects of the agent on military-aged Iranian veterans following battlefield exposure. It was found that total and free testosterone levels were markedly decreased in the first 5 weeks after exposure. LH and FSH increased by the third and fifth week, respectively. All hormones had returned to normal by the 12th week after exposure. In 28 (66%) out of the 42 men examined 1 – 3 years after injury, the sperm count was less than 3 million cells/mL and the FSH level was higher compared with that of normal men.[112] A study of 77 SM-exposed veterans, 3 – 9 years after exposure, also revealed a significantly diminished sperm count and motility in comparison to unexposed veterans.[74]

While results of these aforementioned investigations strongly suggest that mustard exposure might have adverse effects on (at least) male fertility, results of another study by Ghanei et al (2004) failed to reveal an association between infertility and SM exposed residents of Sardasht.[113]

Neuropsychiatric complications

A. Central nervous system

Casualties from WWI and from the Iran-Iraq conflict were noted to have long-term mood and anxiety disorders, as well as posttraumatic stress disorder (PTSD).[52, 62] Debility, loss of vitality, impaired concentration, sensory hypersensitivity, diminished libido, weakened potency, neuralgic complaints, and disorders in autonomic regulation

of the heart have been reported in German poison gas workers during WWII.[114, 115] Neuropsychiatric evaluation of 1428 Iranian veterans, 3 – 9 years after exposure to SM, revealed anxiety (15%), depression (46%), personality disorders (31%), convulsions (6%), and psychosis (3%).[74] Disorders of consciousness (27%), attention (54%), emotion (98%), behavior (80%), thought process (14%), and memory (80%) were reported, 3 – 5 years after exposure, by Tabatabai and colleagues (1988), who studied 70 SM-poisoned patients.[116] In another study, decreased libido and impotence were recorded in 52% and 9% of the patients, respectively. Quite interestingly, 10% of the patients revealed an increased libido.[117] Functional photophobia, functional aphonia, and effort syndrome have also been reported.

B. Peripheral nervous system

A frequent long-term complication in patients exposed to SM is delayed neuropathic symptoms, which were underrepresented in most previous studies.[118] Balali-Mood and colleagues carried out electromyography (EMG) and nerve conduction velocity (NCV) on 40 Iranian veterans with severe late manifestations of SM poisoning. Seventy-seven point five percent of the patients revealed abnormalities in the peripheral nervous system. 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 6 (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.[65]

Laboratory diagnosis

Alkylation products of SM with DNA and proteins (e.g. hemoglobin and albumin), as well as its urinary metabolites, have proven useful targets for 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 of exposure to SM, possibly up to several months.

A. Determination of urinary metabolites of SM

While the hydrolysis product of SM, namely thiodiglycol, is only a minor metabolite, the sulfoxide derivative of thiodiglycol is abundantly present in urine[119] and can be reduced to thiodiglycol for gas chromatography-mass spectrometry (GC-MS) analysis.[132] Unfortunately, both thiodiglycol and its sulfoxide are not unequivocal markers of poisoning in humans and low concentrations are present in normal human urine.[120 – 122]

The β-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.[123] 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 β-lyase metabolites were detected in one sample collected 13 days after the alleged SM exposure.[123, 124]

B. Determination of SM adducts with DNA

The primary site of DNA alkylation by SM is the N7 position of deoxyguanosine residues.[125] Upon depurination of the resulting N7-(2-hydroxyethylthioethyl0-2′-deoxyguanosine, N7-(2-hydroxyethylthioethyl) guanine (N7-HETE-Gua) is obtained. While GC-MS analysis proved problematic, N7-HETE-Gua could be conveniently analyzed, using the liquid chromatography- mass sepectrometry (LC-MS).[126] 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) was successfully developed, using monoclonal antibodies raised against N7-HETE-guanosine-5′-phosphate coupled to keyhole limpet hemocyanin.[127] 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.[128] The ELISA was also successfully applied in toxicokinetic studies in which levels of adducted DNA were followed in conjunction with measurement of intact SM.[129]

C. Determination of SM adducts with proteins

The alkylation of proteins by SM mainly occurs in carboxyl, α-amino, and sulfhydryl groups, as well as in the nitrogens of the imidazole ring of histidine.[24, 16] Definitive evidence of specific alkylation sites can be obtained by using modern mass spectrometric (MS) techniques. While MS methods can be used to confirm the diagnosis under more sophisticated conditions, the ELISA approach was mainly developed for use under field

conditions. Hemoglobin and albumin are two abundant proteins in human blood that can be readily isolated for determination of SM adducts.[130]

With hemoglobin, the adducts with amino function in N-terminal valine of the α- and β-chains have proved to be the most convenient for diagnosis. These adducts are generally stable (in contrast to DNA damage, which may be repaired within days) and have the same life span in humans (approximately 120 days) *in vivo* as the native proteins. Consequently, the adducts may be detectable for long periods of time after the actual exposure. In the case of chronic exposure, the adducts will accumulate in time.[130] The N-terminal valine adduct has been detected in samples from casualties of accidental exposure to SM[131] and from Iranian CW casualties taken 22 – 26 days after the alleged exposure.[128]

A sensitive method for analysis of the SM adduct, in terms of the cystein-34 residue, in human serum albumin has been developed and successfully applied to samples from Iranian casualties of the Iran-Iraq War, who were allegedly exposed to SM, 8 – 9 days before sampling. Compared to the assay for analysis of N-terminal valine adduct, it could be expected that this assay is less retrospective, due to the faster elimination rate of albumin adducts.[130]

Management

Management is divided into three parts: first aid measures, triage, and medical treatment.

I. First aid

1- Casualties must be quickly removed from the contaminated area by adequately protected attendants.[132, 133]

2- All contaminated clothes must be removed and destroyed.

3- All the skin surface should be washed out thoroughly by showering and using neutrogenic soap (pH around 7.0). Washing of the affected area with oil, kerosene, or gasoline, followed by washing with soap and water, has also been advocated.[52, 54]

4- In the case of liquid contamination, the eyes should be immediately rinsed out, using generous amounts of normal saline, Ringer solution, or water from any source.[132, 133]

5- Reassurance and transferring the casualties to the medical clinic/site for triage would be the next step.

II. Triage

The casualties should be quickly examined by medical doctors for the severity grading of SM intoxication as mild, moderate, and severe. The moderately and severely intoxicated patients must be transferred to the nearest medical center for emergency management. The mild cases who may not reveal any sign of intoxication should be under observation for at least 24 hours. The patients showing any sign of SM poisoning during observation should also be transferred to the medical center. The asymptomatic patients may be discharged after 24 hours.

III. Medical treatment

Medical treatment of SM poisoning may be divided into three parts: antidotal treatment, general treatment, and specific organs' care.

A. Proposed antidotal treatment

1- Five hundred mg sodium thiosulfate per kilogram body weight should be administered as soon as possible. Sodium thiosulfate reacts with mustards, when these agents are in the cyclized form and is thus an effective antidote against systemic intoxication by SM, especially when taken before exposure.[134 – 136] It can also be combined with a number of other drugs such as cystine, sodium citrate, dexamethasone, promethazine, heparin, and vitamin E, to increase its protective activity against SM.[136, 137]

B. General treatment

1- Pain management and sedation of the severely intoxicated patients may be required.

2- Reassurance and tensive love care of the patients are very important in their management.

The application of extracorporeal detoxification procedures, such as hemoperfusion and hemodialysis, is without any proven therapeutic or clinical effect, as no active mustard has been identified in blood taken from victims. Moreover, these procedures are not without danger, facilitating coagulation disorders and systemic infections in these immunocompromised patients.[40]

C. Specific organs' care

C.1. Management of skin lesions

1- The skin should be washed out with 0.2% or 0.3% chloramine-T solution, at least six times a day.[138] There is no proof that chloramine applied to the skin reacts with mustard, but because of its disinfectant propertie chloramine might be useful.

2- Frequent dressings of silver sulfadiazine (Flamazine) 1% cream should be applied to prevent secondary infections.[19, 138, 139]
3- Calamine lotions and local steroid solutions can be used for erythema and minor blistering to reduce itching and irritation.[8, 139, 140]
4- Systemic analgesics from paracetamol to morphine, and systemic antihistamines (e.g. promethazine) or sedatives (e.g. barbiturates) can be helpful in severe cases with intense pain and itching.[19, 49, 139]
5- Small blisters (< 2 cm) should be left intact and not be debrided. However, once they have ruptured spontaneously, then debridement should be considered to accelerate healing.
6- Blisters larger than 2 cm must be debrided and opened while being lavaged with normal saline and treated topically with mafenide acetate (Sulfamylon) cream or silver sulfadiazine cream. Blister fluid must be suctioned before debridement.[8, 23]
7- Skin grafting should be considered for large full-thickness burns.[8, 140] Several days after exposure, removal of the surface of the skin in the affected area until capillary bleeding occurs (dermal abrasion) may also hasten recovery.[141, 142]

Treatment in the chronic phase is mainly symptomatic. Systemic antihistamines and local emollients can help to reduce itching and improve skin dryness. Frequent baths should be discouraged in these patients. Sunscreen lotions or creams can also be applied for hyperpigmented lesions. Contractures rarely occur with chemical burns caused by SM.

C.2. Management of eye lesions

1- The eyes should be washed out as soon as possible even in the asymptomatic patients. Because of the rapid and irreversible reaction of SM with ocular tissues, it may seem useless to start irrigation more than 10 – 15 minutes after exposure. Several different solutions have been recommended for this purpose, including pure water, normal saline, 1.5% sodium bicarbonate, or 0.5% dichloramine-T.[93, 143] Saturated solutions of sodium sulfate or magnesium sulfate, as well as zinc or boric acid,[143] have also been suggested. However, among all these fluids, none has proved more effective than tap water.
2- Petroleum jelly could be applied on the follicular margins to prevent sticking. However, its use should be delayed until some time after exposure to avoid mustard concentration in this oily moiety.[144, 145]
3- Mydriatics should be used to ease the eye pain produced by spasm of the ciliary muscle and to prevent posterior iridolenticular adhesions.
4- Local anesthetic drops should be avoided other than for ophthalmologic examination, as they are toxic to both healthy and damaged corneas.[146]
5- Local steroids should generally be avoided, especially when there is evidence of corneal epithelial defects. After epithelial defects are healed, however, they can be used to reduce chemosis and corneal epithelial edema.[95]
6- Ocular pads and bandages should be avoided as they might raise the temperature and accelerate the toxic effects.[93]
7- Dark glasses and reassurance to the patient are very important, as eye lesions produce severe photophobia and fear.[34, 145]

To date, there has been no definite treatment for the delayed keratitis caused by SM. However, artificial tears,[95] therapeutic contact lenses,[34, 47, 91, 92] local/systemic corticosteroids, and other immunosuppressive drugs such as azathioprin, may be used according to the severity of keratitis.[95, 96] Corneal argon laser photocoagulation has proven ineffective in the prevention of corneal vascularization.[95] Keratoplasty has also limited success, since the limbal blood supply is poor in these patients.[92, 95]

C.3. Management of the respiratory toxic effects

1- Physiotherapy, oxygen, and assisted ventilation are the mainstays of treatment.[40]
2- Inhalation of moist air and mucolytics such as acetylcysteine have been used in the treatment of Iranian casualties, although their efficacies are lacking.[148]
3- Antibiotic cover is recommended in view of the risk of secondary infections.[19, 40]
4- Immediate inhalation of beclomethasone in large doses has been suggested in order to prevent lung edema after contamination. Taking five deep breaths of the drug every 10 minutes is the preferred therapy.[23]
5- Bronchodilators have been shown to be helpful in patients with increased airway hypersensitivity. Combination of a β-agonist (e.g. salbutamol) and an anticholinergic (e.g. ipratropium bromide) has been found to be more effective than any of the other bronchodilators used alone.[78]
6- In cases of very severe respiratory damage, chemical pneumonitis could occur and may demand intensive care therapy.[19]

C.4. Management of the bone marrow depression

Bone marrow depression leading to leukopenia and aplastic anemia should be treated with granulocyte, platelet, and red cell transfusions. Granulocyte colony stimulating factor and other related factors should be considered in severe leukopenia, although their efficacies have not been proved in SM poisoning.[8, 19, 139] Bone marrow transplants have not been attempted, but may be successful.

Conclusion

The widespread use of SM as an incapacitating warfare agent in the past century has proved its highly long-lasting toxic effects. This experience may ensure further use of the agent in future military conflicts and terrorist attacks. SM exerts its toxicity through a number of postulated pathogenic mechanisms including DNA alkylation, NAD depletion, and inactivation of glutathione. Eyes, skin, and the respiratory system are the three major targets for the local irritant 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 neurologic and digestive disorders. Even more important is a wide range of delayed toxic 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 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 human subjects who have been exposed to the agent are required. Immunological and psychological dysfunctions, as well as SM relationship to carcinogenesis and teratogenesis are important fields which are underrepresented in previous investigations. There is also paucity of information regarding the medical management of acute and delayed toxic effects of SM poisoning, a subject which greatly challenges health-care specialists.

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