Sulfur mustard toxicity: history, chemistry, pharmacokinetics and pharmacodynamics

Article *in* Critical Reviews in Toxicology · May 2011

CITATIONS
42

READS
135

6 authors, including:

Kamyar Ghabili
Yale University
186 PUBLICATIONS **1,322** CITATIONS
SEE PROFILE

Mostafa Ghanei
Baqiyatallah University of Medical Sciences
363 PUBLICATIONS **4,191** CITATIONS
SEE PROFILE

Khalil Ansarin
112 PUBLICATIONS **1,118** CITATIONS
SEE PROFILE

Yunes Panahi
Baqiyatallah University of Medical Sciences
252 PUBLICATIONS **2,479** CITATIONS
SEE PROFILE

Critical Reviews in Toxicology, 2011, 1–19, Early Online

informa
healthcare

REVIEW ARTICLE

Sulfur mustard toxicity: History, chemistry, pharmacokinetics, and pharmacodynamics

Kamyar Ghabili,[1] Paul S. Agutter,[2] Mostafa Ghanei,[3] Khalil Ansarin,[1] Yunes Panahi,[3] and Mohammadali M. Shoja[4]

[1]Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, [2]Theoretical Medicine and Biology Group, Glossop, Derbyshire, UK, [3]Research Center of Chemical Injuries, Baqiyatallah University of Medical Sciences, Tehran, Iran, and [4]Department of Ophthalmology, Indiana School of Medicine, Indiana University, Indianapolis, Indiana, USA

Abstract

Sulfur mustard (SM) and similar bifunctional agents have been used as chemical weapons for almost 100 years. Victims of high-dose exposure, both combatants and civilians, may die within hours or weeks, but low-dose exposure causes both acute injury to the eyes, skin, respiratory tract and other parts of the body, and chronic sequelae in these organs are often debilitating and have a serious impact on quality of life. Ever since they were first used in warfare in 1917, SM and other mustard agents have been the subjects of intensive research, and their chemistry, pharmacokinetics and mechanisms of toxic action are now fairly well understood. In the present article we review this knowledge and relate the molecular-biological basis of SM toxicity, as far as it has been elucidated, to the pathological effects on exposure victims.

Keywords: *Chemical weapon; mechanism; molecular; sulfur mustard; toxicity*

Contents

Address for Correspondence: Mohammadali M. Shoja, MD, Department of Ophthalmology, Indiana School of Medicine, Indiana University, Indianapolis, IN 46202, USA. E-mail: shoja.m@gmail.com

(Received 09 June 2010; revised 02 November 2010; accepted 15 November 2010)

ISSN 1040-8444 print/ISSN 1547-6898 online © 2011 Informa Healthcare USA, Inc.
DOI: 10.3109/10408444.2010.541224

1. Introduction

"Mustards" are a group of compounds with the general structure R-CH_2CH_2X, where R is a leaving group and X is a Lewis base. Many are bifunctional: $(R\text{-}CH_2CH_2)_2X$. Their range of toxic effects on humans and other animals accounts for their use in warfare and, occasionally, in cancer therapy. Figure 1 shows examples. This review will focus on the mustard agent that has been used most frequently for military purposes, bis(2,2′-chloroethyl)thioether (R = Cl, X = S), commonly known as sulfur mustard (SM); it also has several other synonyms (Figure 1). However, many of the observations and inferences we discuss apply to mustard agents in general. Some investigations into the biological effects of SM have involved animal experiments using the monofunctional compound 2-chloroethyl ethyl sulfide (CEES), which has a lower acute toxicity than SM; others have involved SM itself, or nitrogen mustards.

The article is divided into three main sections:

1. History—The development of SM and other mustard compounds, their use in warfare, and overview of the pathological effects of SM exposure.
2. Physical and chemical properties of SM and its general biological effects.
3. Molecular basis of SM-related pathologies.

2. History

2.1. Development and synthesis of SM

The historical development of SM was described by West (1919), Jackson (1936), and Medema (1986). Depretz allegedly synthesized the compound from ethene and sulfur dichloride in 1822 and Riche did likewise in 1854:

$$SCl_2 + 2\,CH_2{=}CH_2 \rightarrow (Cl\text{-}CH_2CH_2)_2S$$

but neither chemist mentioned its vesicant properties. Niemann (1860) repeated the procedure and his account of the vesicant effect was unequivocal: "They [the vesicant properties] are represented by the fact that even traces brought into contact with the skin at first cause no pain, but after several hours result in a reddening of the skin, and later blisters from burns, which fester for a long time and heal very badly, leaving severe scars." Guthrie (1859, 1860) gave a similar account. In 1886, Meyer devised a two-stage synthetic process that gave a higher yield, mentioned, e.g., in the obituary by Richardson (1897):

$$2\,(HO\text{-}CH_2CH_2\text{-}Cl) + K_2S \rightarrow (HO\text{-}CH_2CH_2)_2S + 2KCl$$

$$3\,(HO\text{-}CH_2CH_2)_2S + 2PCl_3 \rightarrow 3(Cl\text{-}CH_2CH_2)_2S + 2H_3PO_3$$

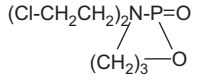
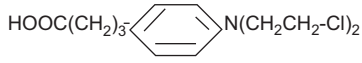
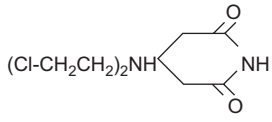
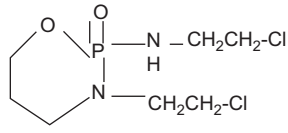
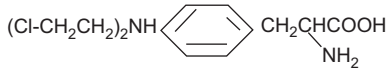
Compound	Synonyms	Structure
Sulfur mustards		
Bis(2,2'chloroethyl)thioether (mustard gas)	Sulfur mustard, Yperite, HD, LOST, MG	$(Cl\text{-}CH_2CH_2)_2S$
1,2-Bis(2-chloroethylthio)ethane	Sesquimustard, Q	$Cl\text{-}CH_2CH_2\text{-}S\text{-}CH_2CH_2\text{-}S\text{-}CH_2CH_2\text{-}Cl$
1,3-Bis(2-chloroethylthio)-n-propane	(Note: military stockpiles also contain the butane and pentane' analogues)	$Cl\text{-}CH_2CH_2\text{-}S\text{-}CH_2CH_2CH_2\text{-}S\text{-}CH_2CH_2\text{-}Cl$
2-chloroethylchloromethylthioether		$Cl\text{-}CH_2CH_2SCH_2\text{-}Cl$
Bis(2-chloroethylthio)methane		$(Cl\text{-}CH_2CH_2S)_2\text{-}CH_2$
Bis(2-chloroethylthiomethyl)ether		$(Cl\text{-}CH_2CH_2SCH_2)_2O$
Bis(2-chloroethylthioethyl)ether	O mustard	$(Cl\text{-}CH_2CH_2SCH_2CH_2)_2O$
Nitrogen mustards: military use		
Bis(2-chloroethyl)ethylamine	HN1	$(Cl\text{-}CH_2CH_2)_2NHCH_2CH_3$
Bis(2-chloroethyl)methylamine	HN2, Mechlorethamine, Chlormethine, Mustine, Nitrogen mustard, Mustargen	$(Cl\text{-}CH_2CH_2)_2NHCH_3$
Tris(2-chloroethyl)amine	HN3	$(Cl\text{-}CH_2CH_2)_3N$
Nitrogen mustards: therapeutic use		
Cyclophosphamide	Endoxan, Cytozan, Neosar, Procytox, Revimmune, Cytophosphane	
Triscarboxymethylphenyl-bis(2-chloroethy)ethylamine	Chlorambucil	
5-[Bis(2-chloroethyl)amino]-1H-pyrimidine-2,6-dione	Uramustine	
N-3-bis(2-chloroethyl)-1,3,2-oxazaphosphinan-2-amide-2-oxide	Ifosfamide	
4-[Bis(chloroethyl)amino] phenylalanine	Mephalan, Alkeran	

Figure 1. Compound, synonyms, and structure of different mustards used in warfare and cancer therapy.

Later, Hans Thacher Clark and Emil Fischer modified the Meyer process by replacing phosphorus trichloride with HCl in the reaction with thiodiglycol (Vickery, 1975):

$$(\text{HO-CH}_2\text{CH}_2)_2\text{S} + 2\text{HCl} \rightarrow (\text{Cl-CH}_2\text{CH}_2)_2\text{S} + 2\text{H}_2\text{O}$$

Clarke was apparently hospitalized for 8 weeks because of the burns he suffered when a flask broke during this process. Fischer's subsequent report about the incident to the German Chemical Society allegedly inspired the first deployment of SM as a chemical weapon (Duchovic and Vilensky, 2007).

2.2. Use in warfare

In September 1917 German military first used SM at Ypres in the course of the First World War; from this, the old name "Yperite" originated (Jacques, 1991). Upon deployment on the battlefield, SM resulted in many casualties among enemy forces, although the effects usually became evident only around 12 hours after exposure. In cases of fatality, death typically succeeded some 4–5 weeks later. Four thousand British armed forces deaths and 16,526 nonfatal injuries were thenceforth due to SM in the War (Gilchrist, 1928). More generally, only very high doses (dermal exposure at 64 mg/kg or inhalation at 1500 mg min/m^3) are lethal to humans within approximately 1 hour (Marshall, 1987).

Since the First World War, SM has been deployed in numerous combats, often against civilians (Feakes, 2003; Lyon, 2008): the United Kingdom against the Red Army (1919), Spain against Rif revolutionaries in Morocco (1921–1927), Italy in Libya (1930), the Soviet Union against Japan in Xinjiang (1930s), Italy against Abyssinia (1935–1940), Poland against Germany, Germany against Poland and the Soviet Union, and Japan against China during the Second World War, Egypt against North Yemen (1963–1967), Iraq against Iran (1983–1988), Armenians against the Azerbaijanis in the Nakhchivan (1992), and Sudan against insurgents (1995–1997). Regrettably, Sardasht, a small city in Northweatern Iran, was the world´s first city in which the civilians were attacked with chemical weapons (Khateri and Wangerin, 2008). SM has been used in experiments on military volunteers as well (Goodwin, 1998).

2.3. Overview of the pathological effects of SM exposure

A comprehensive review of earlier literature about mustard compounds was published by Gray (1989) and clinical considerations were discussed by, e.g., the World Health Organization (1970), Sidell and Hurst (1992), and Kehe and Szinicz (2005). The Institute of Medicine (1993) reported evidence for causal relationships between SM exposure and a wide range of health conditions.

The acute effects of SM exposure are usually delayed (Kehe et al., 2009b); there are usually no signs or symptoms during the first hour, though occasional cases of nausea, vomiting, and eye irritation have been reported, and contact with very high doses may lead to convulsions and coma within this period. The delay is shorter with liquid than with vapor contamination. Within 2–6 hours of exposure, typical signs are nausea, fatigue, headache, painful eye inflammation with photophobia, reddening of face and neck, soreness of throat, tachycardia, and increased respiratory rate. These symptoms become more severe during the subsequent 6–24-hour period, with skin inflammation and blistering. The blistering becomes more marked during the following 24 hours, and there is productive coughing with pus and necrotic sloughed epithelial material. Anemia and neutropenia may become apparent after 4–5 days, indicating bone marrow dysfunction; this effect may possibly be reversed by treatment with granulocyte colony-stimulating factor (Meisenberg et al., 1993; Anderson et al., 2006). In severe cases, death is probable after a delay of days or weeks. In less severe cases, the burns heal slowly, but as with other types of burn there is a risk of infection, resulting in sepsis (Institute of Medicine, 1993). The other major system affected is the respiratory tract, and it is here that most of the chronic consequences of SM exposure are noticeable, with associated morbidity and mortality. We have reviewed the acute and chronic effects on lungs and other organs elsewhere (Ghabili et al., 2010). A thorough discussion of the pathological changes (acute and chronic) in lungs following SM exposure has been also published by Ghanei et al. (2008), Ghanei and Harandi (2007), and Beheshti et al. (2006).

3. Physical and chemical properties of SM relevant to biological effects

3.1. Physical properties

In its pure form, SM (relative molecular mass 159.1) is a colorless, viscous liquid at room temperature, with a specific gravity of 1.27, a melting point of 14°C, and a boiling point of 218°C at 1 atmosphere pressure (it decomposes before boiling). Its vapor pressure is 0.11 mm Hg at 25°C. The vapor is denser than air, so on release it tends to accumulate near ground level. It is fat soluble rather than water soluble (the water solubility is 0.06% at 20°C), so it is rapidly absorbed by the skin (Ivarsson et al., 1992). It persists in the environment and remains active underground for up to 10 years

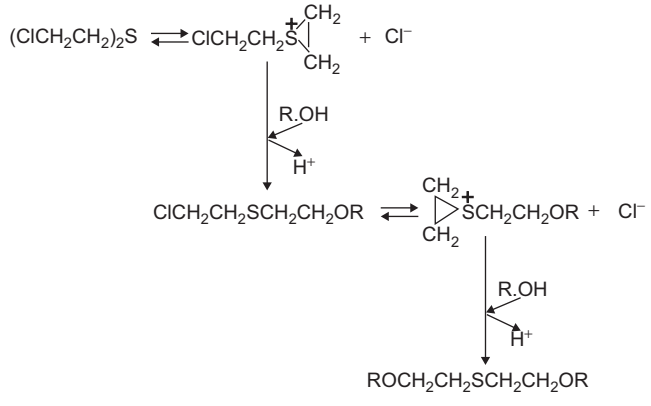


Figure 2. Reaction of MG SM via sulfonium ion intermediate. If R = H, this represents a two-stage hydrolysis of SM; the final product is thiodiglycol.

and (depending on weather conditions) even on the soil surface for several weeks, with significant environmental consequences (Beck et al., 2001; Ashmore and Nathanail, 2008; Medvedeva et al., 2008). Other mustard agents differ in melting point and other physical properties but have broadly similar biological effects.

3.2. Chemistry of SM

The chemistry of SM has been discussed in detail by Reid (1958) and Ross (1962). Mustards often react by undergoing internal cyclization to form onium ions (e.g., sulfonium, aminium); the leaving group is one of the chlorine atoms. SM, for example, forms a three-membered cyclic sulfonium group, an electrophile that attacks and alkylates many biomolecules and is susceptible to slow hydrolysis under physiological conditions (Figure 2). In a bifunctional mustard such as SM, both chlorines can act as leaving groups in separate reactions.

3.2.1. Hydrolysis and reaction with alcohols

Figure 2 shows a schematic diagram of the reaction of SM with an alcohol or with water (R = H) involving cyclic sulfonium intermediates. The agent can therefore chemically modify carbohydrates and hydroxy groups on protein side chains. In alkaline solution the half-life for hydrolysis is about 5 minutes, but the process is slower at lower pH. Various catalysts have been investigated for accelerating the hydrolysis of SM in the environment (Cerny and Cerny, 1997).

The intramolecular cyclization of SM to the sulfonium ion intermediate is facilitated by heat in an aqueous environment. This may explain why warm and moist regions of the body (mucous membranes, eyes, respiratory tract, etc.) are especially vulnerable to the acute toxic effects of the agent (Ward and Seider, 1984; Somani and Babu, 1989).

3.2.2. Reaction with thiols

Thiols undergo an analogous alkylation reaction of the general kind:

$$\text{R-SH} + (\text{Cl-CH}_2\text{CH}_2)_2\text{S} \rightarrow \underset{\text{HCl}}{\text{R-SCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{-Cl}} +$$

The other chlorine atom can react similarly with another molecule of the same thiol or a different one, leading to cross-linking:

$$\underset{\text{HS-R}}{\text{R-SCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{-Cl}} + \rightarrow \underset{\text{HCl}}{\text{R-SCH}_2\text{CH}_2\text{SCH}_2\text{CH}_2\text{S-R}} +$$

3.2.3. Oxidation

The sulfonium salts contribute significantly to the biological activities of SM, but oxidation products may also be important (Francis et al., 1957). The sulfoxide $[\text{OS}(\text{CH}_2\text{CH}_2\text{Cl})_2]$ reacts much more slowly than the sulfone $[\text{O}_2\text{S}(\text{CH}_2\text{CH}_2\text{Cl})_2]$, so oxidation of SM to the sulfoxide constitutes detoxification (Riches et al., 2007). The sulfone, however, is susceptible to nucleophilic attack (Figure 3), which makes it toxic; it is a vesicant, like the parent compound. Rats that have received intravenous SM produce conjugates of the sulfone in their urine (Black et al., 1992).

3.2.4. Reactions with amines

SM reacts with ammonia and with primary, secondary and tertiary amines to yield a variety of products (Figure 4). It can therefore chemically modify proteins and phospholipids.

3.2.5. Reactions with purine and pyrimidine bases

At neutral pH, SM alkylates purine and pyrimidine bases in nucleosides, nucleotides, and nucleic acids, preferentially at N-7 of guanine and N-1 of adenine (Wheeler, 1962; Walker, 1971; Ball and Roberts, 1972; Ludlum et al., 1986). Reactions with O-6 and N-2 of guanine and N-6 of adenine have also been reported (Ludlum et al., 1984, 1986; Habraken and Ludlum, 1989). Figure 5 shows examples of products isolated from the reaction of SM with guanine residues in DNA.

3.3. Biological effects

Apart from its injurious effects on the respiratory tract and its vesicant action (Devereaux et al., 2002; Evison et al., 2002), SM is a mutagen and a potential carcinogen. An issue of the *Annals of the New York Academy of Sciences* in 1969 was devoted to this topic. Because of its chemical properties, SM reacts with proteins, RNA, and phospholipids, but its most important cytotoxic action arises from DNA alkylation and cross-linking (Boursnell et al., 1946; Davison et al., 1961; Wheeler, 1962, 1967; Papirmeister and Davison, 1964; Kohn et al., 1965; Lawley and Brookes, 1965; Price et al., 1968; Papirmeister et al., 1969, 1970, 1984a, 1984b; Roberts et al., 1971; Walker, 1971; Ball and Roberts, 1972; Gross et al., 1981; Meier et al., 1984; Ludlum et al., 1986; Habraken and Ludlum, 1989). Even low-dose exposure is likely to generate interstrand DNA cross-links, which are lethal to proliferating cells especially during late G1 or S phase (Mauro and Elkind, 1968; Roberts et al., 1968, 1986; Ludlum et al., 1978).

High doses may produce rapid cell death by other mechanisms, which are probably implicated in acute injuries to the cornea, mucous membranes, and skin. One such mechanism is NAD depletion (Gross et al., 1985; Papirmeister et al., 1985). Poly(ADP-ribose) polymerase is activated by DNA strand breaks, such as those produced by sulfur and nitrogen mustards, and the activated enzyme quickly depletes cellular NAD pools. The consequent lowering of intracellular ATP levels can cause rapid cell death.

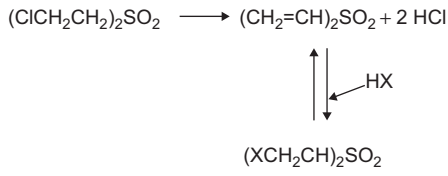


Figure 3. Addition reaction by sulfone derivative of SM. HCl is eliminated from the sulfone to yield divinylsulfone, which is susceptible to nucleophilic attack by the moiety X.

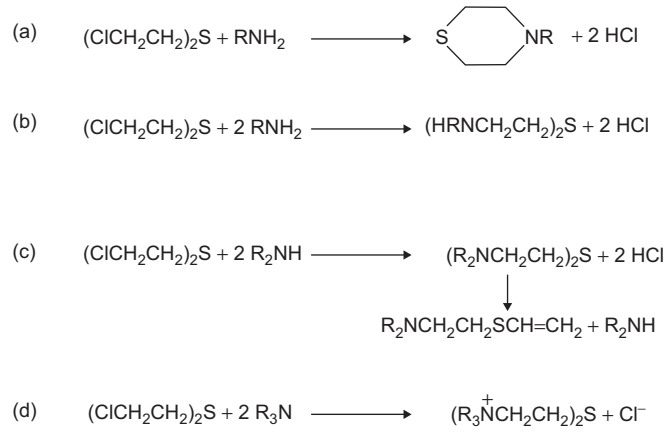


Figure 4. Reactions of SM with amines. One molecule of SM may react with either one (a) or two (b) molecules of ammonia or a primary amine; or with two molecules of a secondary amine (c), one of which may subsequently be eliminated; or with two molecules of a tertiary amine (d) to generate a quaternary ammonium derivative.

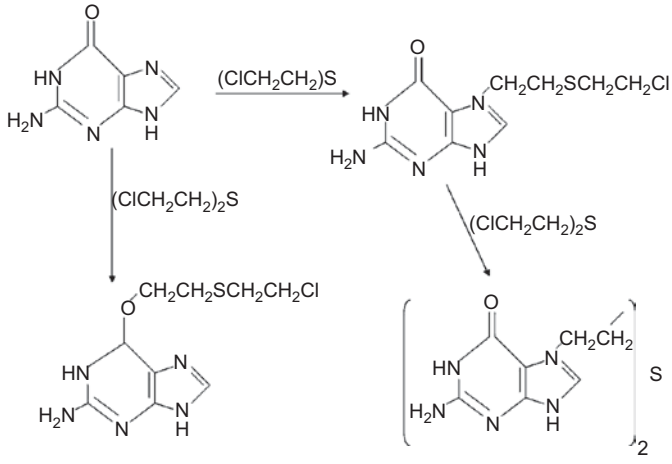


Figure 5. Reactions of SM with guanine. SM can react with adenine and with pyrimidines, but guanine residues in nucleic acids are especially susceptible. Reactions with $O\text{-}_6$ and $N\text{-}_7$ of the purine derivative are shown; the bifunctional agent can cross-link two guanine residues, for example via their N_7 atoms as illustrated. Reactions with the $N\text{-}_2$ atom have also been reported.

Another well-established mechanism is the inactivation of sulfhydryl-containing proteins (Byrne et al., 1996) and peptides such as glutathione (GSH) (Figure 6). This has implications for the redox state of the cell internum, leading to lipid peroxidation, membrane damage, calcium imbalance, and cell death (Langford et al., 1996).

The molecular basis of SM toxicity will be explored in greater detail in Section 4.

3.4. Metabolism and excretion

The usual routes of entry are the skin, respiratory tract, and eyes, or through the gastrointestinal tract if SM-contaminated food is consumed. Some 80% of SM applied to the skin evaporates, 10% remains in the skin, and 10% is absorbed systemically (Renshaw, 1946) and is distributed among various tissues (Drasch et al., 1987; Hambrook et al., 1993). After intravenous (IV) injection

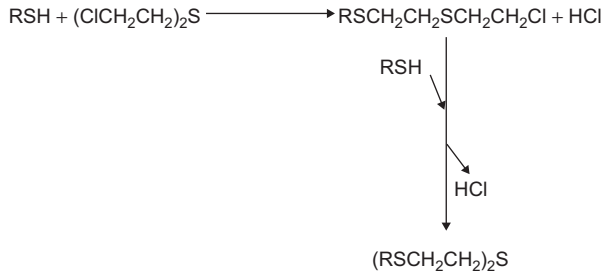


Figure 6. Reactions of SM with sulfhydryl compounds. The sulfhydryl compound is S-alkylated, and proteins containing thiol groups may be internally or extrinsically cross-linked.

into rabbits, SM was distributed throughout the body and was concentrated in the liver, kidneys, and lungs; about 20% was excreted within 12 hours (Boursnell et al., 1946) and most of it had been excreted after 72 hours (Davison et al., 1961). In dogs, equilibration between blood and tissues is attained within 5 minutes of perfusion of the lungs (IARC, 1975).

Somani and Babu (1989) described the toxicodynamics of SM, a subject of research since the Second World War. Acute LD_{50} values vary markedly among species and in many cases are uncertain; in rats they are reported to be 9 mg/kg percutaneous and 100 mg/m^3 for 10 minutes by inhalation. In female mice, the LD_{50} values were determined during a 14-day observation to be 5.7–8.1 mg/kg percutaneous, 23 mg/kg subcutaneous (Vijayaraghavan et al., 2005; Sharma et al., 2008), 8.1 mg/kg oral (Vijayaraghavan et al., 2005), and 42.3 mg/m^3 for 1 hour by inhalation (Lakshmana Rao et al., 1999). Additionally, the 24-hour inhaled LD_{50} was calculated as 995 μg/kg in a guinea pig model (Allon et al., 2009). A comprehensive review of the toxicity of the vesicant agents including varying LD_{50} values in several species by multiple routes of administration was published by Watson and Griffin (1992). The physiological half-life may also vary among species; in rat it is reportedly 7.4 days (Hambrook et al., 1993). The urinary metabolites include thiodiglycol and its conjugate (15%), glutamine-bis(β-chloroethyl sulfide) conjugates (45%), glutamine-bis(β-chloroethyl sulfone) conjugates (7%), and bis(β-chloroethyl sulfone) and conjugate (8%), with traces of cysteine conjugates (Black et al., 1992). These findings indicate that hydrolysis, oxidation, and reactions with amines and thiols occur physiologically. Similar results have been obtained from rodents after intraperitoneal injection (Roberts and Warwick, 1963). The parent compound can be detected in urine for up to a week after exposure (Vycudilik, 1985).

3.5. Mutagenicity and genotoxicity

Because it modifies DNA (especially guanine residues) and causes cross-linking, SM induces chromosome aberrations in many cell types; it was the first agent shown to cause chromosome abnormalities in Drosophila melanogaster (Auerbach, 1943). The DNA modification is dose related and the spectrum of genetic change, like that caused by X-irradiation, is cell cycle specific (Nasrat, 1954; Sobels and Van Steenis, 1957). Indeed, cytogenetic (chromosome) sensitivity to SM parallels that of X-rays in certain cell lines (Scott et al., 1974).

Subsequent studies demonstrated that SM induces chromosome aberrations in Vicia faba and marsupial lymphocytes (Scott and Bigger, 1972). Fishermen exposed to SM (when they netted leaky barrels of mustard agents that had been dumped at sea after the Second World War) showed elevated sister-chromatid exchange (SCE) frequencies in their peripheral blood lymphocytes (Wulf et al., 1985); DNA alkylation induces SCEs in animal cells (Kaina, 1998).

SM and related compounds also induce mutations in Drosophila (Auerbach and Robson, 1946, 1947; Luening, 1951; Sobels, 1962; Fahmy and Fahmy, 1972; Lee, 1975), L5178Y mouse lymphoma cells (Capizzi et al., 1974), Neurospora crassa (Auerbach and Moser, 1950; Jensen et al., 1950; Stevens and Mylroie, 1950), and Salmonella (Ashby et al., 1991). The effect is again dose related and SM has a mutagenic potency comparable to X-rays. One study demonstrated that occupational exposure to SM and Lewisite (manufactured in combination) induces mutations *in vivo* in human lymphocytes at the hypoxanthine phosphoribosyltransferase gene (hprt) locus (Yanagida et al., 1988).

Nevertheless, SM does not appear to be teratogenic (Somani and Babu, 1989; Sasser et al., 1996), though nitrogen mustards are (Schardein, 1985).

3.6. Use of mustard agents in chemotherapy

Because mustard agents are genotoxic and mutagenic, and halt cell cycle progression during late G1 and S phases, they are particularly injurious to proliferating cells, including cancer cells. Nitrogen mustards are less reactive than SM and have different pharmacokinetics (Colvin and Chabner, 1990), but they have similar effects on DNA and cause dramatic tumor regression in Hodgkin′s lymphoma patients (Schneider et al., 1948). Usually, therapeutic nitrogen mustards are administered systemically and repeatedly over weeks or months; application to the skin has also been tried (Micaily et al., 1990). They were first used as chemotherapeutic agents shortly after the Second World War (Gilman and Philips, 1946) and the history of this usage was reviewed by Jones (1998).

It soon became apparent that nitrogen mustards can induce as well as inhibit cancer growth (Boyland and Horning, 1949; Berger et al., 1986). They have been shown to increase the risk for acute nonlymphocytic leukemia (ANL) during treatment of ovarian cancer (Greene et al., 1982) and breast cancer (Fisher et al., 1985), and to act synergistically with other carcinogenic agents (Epstein, 1984; Beckmann and Nordenson, 1986). Approximately 3–5% of patients receiving therapeutic courses of nitrogen mustards and other alkylating agents develop ANL (Tucker et al., 1988), typically 3–9 years after treatment (Blayney et al., 1987), and if the course is prolonged and intensive, the rate may be as high as 30% (Einhorn, 1978). ANL is highly malignant and responds poorly to conventional therapy. The frequency of solid tumors is also increased in these patients (Tucker et al., 1988). One study of Hodgkin′s disease patients treated with alkylating agents showed an estimated 10-year actuarial risk for ANL of

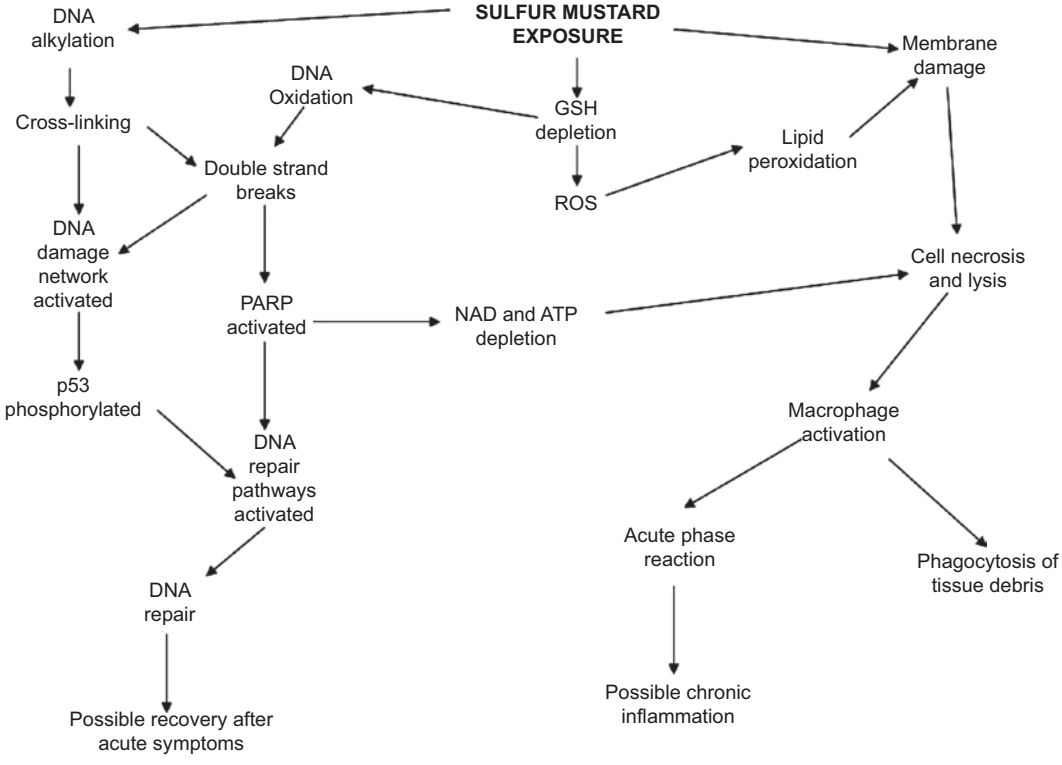


Figure 7. Initial effects of SM exposure on cells. This flow diagram shows some of the major effects of SM on cells: DNA damage, membrane damage, and glutathione oxidation. The consequences of these alterations in biomolecules and cellular structures lead to acute inflammation, which may resolve itself or become chronic, and possibly (if the SM exposure is low- dose and short- lived) to DNA repair. The inflammatory response largely accounts for the early symptoms affecting the exposure victim′s eyes, pharynx, larynx, and skin.

5.9%, for lymphoma 3.5%, and for solid tumors 5.8% (Koletsky et al., 1986).

4. Molecular basis of SM toxicity

In this section we attempt to relate the modifications of biomolecules caused by SM and its analogues (Section 3) to the pathological consequences for the main target organs. Many molecular pathways are involved. The picture remains incomplete, but research conducted particularly during the past decade enables us to outline the major processes as currently understood. In broad terms: (1) SM causes DNA alkylation and cross-linking, protein modification, membrane damage, and GSH depletion (Figure 7); (2) in the target tissues (principally skin, eyes, and respiratory system) there is extensive necrosis, apoptosis, loss of tissue structure, and acute and possibly chronic inflammation. The task is to trace the pathways connecting (1) to (2).

4.1. DNA damage and its consequences

It is generally agreed that the major cytotoxic effect of SM arises from the alkylation of DNA bases and its immediate sequelae, inter- and intrastrand cross-linking and the formation of double-strand breaks. Most cells suffering cytotoxic injury of this kind respond in three ways: poly(ADP-ribose) polymerase (PARP) is activated; the DNA damage network is engaged; and stress genes are up-regulated. Some mammalian cells also release plasminogen activator when their DNA is damaged.

4.1.1. PARP activation

Kehe et al. (2009a) reviewed evidence linking SM-induced DNA damage to PARP activation, cellular nicotinamide adenine dinucleotide (NAD) and adenosine triphosphate (ATP) depletion, and consequent necrotic cell death, which occurs concomitantly with apoptosis (see below; cf. Papirmeister et al., 1985; Das et al., 2003; Chatterjee et al., 2003). PARP engages with strand breaks in DNA through its zinc fingers and is crucial for initiating repair via nonhomologous end-joining and homologous recombination (Vidaković et al., 2005). Its activity increases with increasing levels of DNA damage, so its substrate, NAD, is rapidly depleted when such damage is substantial. When the NAD pool is low, glycolysis is impaired along with other catabolic processes, so the cellular ATP supply is prejudiced. Since ATP is required to replenish the NAD pool, this effect is augmented, and necrosis and cell lysis are likely to ensue. Local and circulating phagocytes, notably macrophages, are then activated.

4.1.2. The DNA damage response network: Involvement of p53

The DNA damage response network triggers cell cycle arrest and DNA repair, or in the case of irreparable damage, inactivation of the cells by senescence or apoptosis (Bartek et al., 2007; Bitomsky and Hofmann, 2009). This may explain why SM is a marginal rather than a potent carcinogen. Central to the network are the protein kinases ataxia-telangiectasia mutated (ATM) and its homologue ATR, and the Mre11/Rad50/Nbs1 (MRN) complex, which activates them (Enoch and Norbery, 1995; Cliby et al., 1998; Paull and Gellert, 1999); ATM is up-regulated by poly(ADP-ribose) (Haince et al., 2007). A particularly important substrate of ATM is p53, which is activated upon phosphorylation and is crucial for activating DNA repair mechanisms. Jowsey et al. (2009) found that when human lymphoblastoid cell lines were challenged with CEES, the phosphorylation of p53 and Chk2 by ATM and ATR was induced and the DNA damage caused by CEES alkylation was repaired by both the base excision repair and nucleotide excision repair pathways. Minsavage and Dillman (2007) showed that p53 in cultured human keratinocytes was phosphorylated on Ser-15 within 15 minutes of SM treatment.

The repair of DNA lesions induced by SM has been studied in systems that are naturally deficient in repair enzymes of the base-excision and nucleotide-excision repair pathways, or had nonfunctional p53 (Walker, 1966; Reid and Walker, 1966, 1969; Lawley and Brookes, 1968; Walker and Smith, 1969; Ball and Roberts, 1970; Walker and Reid, 1971; Plant and Roberts, 1971; Fox and Fox, 1973; Gilbert et al., 1975; Murnane and Byfield, 1981; Savage and Breckon, 1981; Roberts and Kotsaki-Kovatsi, 1986; Roberts et al., 1986). As expected, these repair-deficient cells are particularly sensitive to DNA cross-linking, and they die after exposure to significantly lower doses of SM than are required to kill other cell types. One of the most important findings from these studies is that the DNA repair enzyme O-6-alkylguanine-DNA alkyltransferase has no effect on O-6 derivatives (Ludlum et al., 1986), so the O-6 alkylation products in DNA (Figure 5) may be the most important mutagenic lesions caused by SM and similar compounds.

4.1.3. Stress genes

In human liver carcinoma HepG2 cells, stress gene promoters and response elements associated with DNA and protein damage are activated in a dose- and time-dependent manner by SM (Schlager and Hart, 2000). The consequent activation of second messenger systems and induction of inflammation and oxidative stress are characteristic of chemical injury to this cell line (Tchounwou et al., 2001). Typically, genes for cytochrome P450 isoforms, the xenobiotic response element, growth arrest, and DNA damage proteins such as GADD153 and GADD45 are activated as well as c-*fos*. However, heat shock protein 70 (HSP70) levels are not elevated by SM (Blaha et al., 2000), hence perhaps the greater susceptibility of tissues to alkylation by this agent.

4.1.4. Plasminogen activator release

SM-induced DNA damage may be followed by increased synthesis and release of plasminogen activator (Miskin and Reich, 1980). In skin cells, this is likely to lead to proteolytic degradation of the basal layer of the epidermis, contributing to separation along the dermis-epidermis boundary and consequent skin blistering after exposure to high doses of SM (Miskin and Reich, 1980; Papirmeister et al., 1985; Cowan et al., 1993; Detheux et al., 1997). Plasminogen activator release may also contribute to SM pathogenesis in the

lungs (Liu, 2008). Collagenases and proteoglycanases are also released from activated fibroblasts, but their action is restricted to the site of secretion by various local inhibitors, which have not been well characterized (Woessner et al., 1990). The perturbation of normal tissue structure resulting from the proteolytic degradation of extracellular structures is also likely to stimulate macrophages.

4.2. Other modes of cell injury and their consequences

4.2.1. Protein cross-linking and perturbation of the cytoskeleton

Protein cross-linking, resulting from the alkylation of thiol, hydroxyl, and amino groups by bifunctional agents such as SM, activates the stress genes (see above). *N*-acetylcysteine ameliorates some of the toxic effects the agent, suggesting that thiol alklyation and cross-linking are particularly significant in this regard (Callaway and Pearce, 1958; Fasth and Sorbo, 1973; Vojvodic et al., 1985; Gross et al., 1993; Wilde and Upshall, 1994; Zhang et al., 1995; Paromov et al., 2008). The implication that actin filament disruption is involved in SM-induced cytotoxicity (Dabrowska et al., 1996) was confirmed by Werrlein and Madren-Whalley (2000).

Other elements of the cytoskeleton are also modified by exposure to SM. Keratins are alkylated (van der Schans et al., 2002) and keratin filaments are cross-linked (Dillman et al., 2003), disrupting the intermediate filament network and perturbing cell morphology (Werrlein and Madren-Whalley, 2000). Several type I and II cytokeratins, actin, stratifin, and galectin-7 were found to be significant cytoskeletal target proteins for alkylation by SM in cultured human epidermal keratinocytes (Mol et al., 2008). In addition, cultured keratinocytes exposed to SM showed decreased levels of the keratins associated with cell proliferation in the basal layer of the epidermis (K5 and K14), and increased levels of the keratins associated with terminally differentiated cells in the juxtabasal layer (K1 and K10) (Rosenthal et al., 1998). Changes in mouse ear keratinocyte gene expression after topical application of SM were consistent with these results (Dillman et al., 2006). These authors also found changes in the levels of mRNAs for kinesin family proteins, suggesting that SM might disturb microtubule-based motility, but this has not been confirmed.

4.2.2. Oxidative stress: Oxidants versus antioxidants

The bulk of evidence indicates that oxidative stress, or imbalance between the antioxidant enzymes and products of oxidative reactions, plays a key role in the pathogenesis of both acute and chronic consequences of SM exposure. Intracellular levels of the reduced form of glutathione are markedly depleted by the alkylating effect of SM, and there is a concomitant increase in the levels of reactive oxygen species (ROS) (Han et al., 2004). This has numerous implications for the cell, which will be outlined below. One consequence, however, is lipid peroxidation resulting in membrane damage (Naghii, 2002), another contributor to necrotic cell death and lysis leading to macrophage activation and phagocytosis.

In an in vitro model for the culture of mouse keratinocytes, CEES activated keratinocyte c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein (MAP) kinases and their inhibition negatively affected the expression of the glutathione *S*-transferases GSTP1 and GSTA1-2, respectively (Black et al., 2010). Likewise, CEES-induced lung oxidative injury is attenuated by a metalloporphyrin-based catalytic antioxidant (O´Neill et al., 2010). Twenty-four hours after topical application of a 0.5 LD_{50} dose of SM in rats, significant inhibition of superoxide dismutase, catalase, and glutathione peroxidase activities were noted in the leukocytes and splenic tissue (Husain et al., 1996). In an experimental study, Pohanka and Stetina reported a dose-dependent and bidirectional effect of SM on plasma oxidant levels as estimated by cyclic voltametry; topical application of 20 mg/kg SM for 2 hours increased concentrations of plasma oxidants 24 hours post exposure, whereas application of 80 mg/kg was contrarily associated with a substantial decrease in the plasma oxidants (Pohanka and Stetina, 2009). The authors attributed the latter effect to the cytostatic properties of high-dose SM, which had been previously reviewed by Kehe et al. (2008). Although in their study, plasma antioxidant levels increased with SM doses of 20 and 80 mg/kg, the shift of oxidant-to-antioxidant ratio was predominantly a consequence of oxidants (Pohanka and Stetina, 2009). In another study, intraperitoneal injection of SM (>10–20 mg/kg) was associated with decreased activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione *S*-transferase in the liver as well as depletion of glutathione and increased malondialdehyde levels, a by-product of lipid peroxidation (Jafari, 2007). Increased activity of serum catalase was found in Sardasht victims of SM exposure, possibly indicating an enhanced oxidative load and antioxidant requirements (Shohrati et al., 2008b). Although this study failed to show a significant difference in serum superoxide dismutase activity between the exposed patients and healthy controls (Shohrati et al., 2008b), a later study found diminished levels of enzyme activity among exposed patients with moderate-to-severe pulmonary involvement (Shohrati et al., 2009). Notably, patients with dyspnea and/or chronic cough demonstrated a lower superoxide dismutase activity (Shohrati et al., 2009).

4.2.3. Direct damage to the cell membrane: Macrophage activation, neutrophil recruitment, and acute inflammation

Because it reacts with several functional groups in proteins and lipids, SM also causes direct chemical damage to the cell membrane. Once again, this contributes to cell lysis and local macrophage activity. Membrane damage probably also inactivates the Akt pathway and it may lead to calpain activation via Ca^{2+} influx in some cell types. However, the possible role of these effects in SM-induced apoptosis (see below) has not been established, though SM is known to affect the Akt pathway in Jurkat cells (Zhang et al., 2002).

Different tissues house functionally different macrophage populations, but there are commonalities in their responses. In all affected tissues, the cytotoxic, cytolytic, and extracellular structure–perturbing effects of SM result in the production of superoxide and the secretion of cytokines by macrophages, particularly interleukin (IL)-1, -6, and -8 and tumor necrosis factor-alpha (TNFα). TNFα and superoxide are instrumental in recruiting neutrophils to the site of injury and promoting phagocytosis of injured cells, extracellular structures, and debris (Warren et al., 1990; Strieter and Kunkel, 1994). The ensuing acute phase reaction accounts for the early effects of SM exposure on eyes and skin (Section 4.1). For instance, skin exposed to SM quickly shows infiltration by activated immune cells, up-regulated expression of genes for inflammatory mediators, and increased secretion of those mediators (Rikimaru et al., 1991; Tsuruta et al., 1996; Ricketts et al., 2000; Sabourin et al., 2000, 2002). In particular, TNFα-positive neutrophils accumulate in injured skin within 3 hours (Wormser et al., 2005). Abe et al. (1996) found that an increase in endothelial-leukocyte adhesion molecule 1 (ELAM-1 or E-selectin) during healing of SM skin lesions played a major role in recruitment of the inflammatory cells.

Kehe et al. (2009a) emphasized the activation of inflammatory mediators such as IL-1α, IL-1β, IL-6, IL-8, and TNFα in tissue responses to SM exposure. Phagocytes are not their only sources. Several studies have shown that all these cytokines are up-regulated in normal human keratinocytes after SM exposure (Arroyo et al., 1999; Lardot et al., 1999; Sabourin et al., 2000, 2002, 2004), recruiting cells of the immune system to the site injured by the agent. Inhibition of the MAP kinase (MAPK) family member p38 appears to block this up-regulation in cultured keratinocytes (Dillman et al., 2004). Using a human keratinocyte culture, Pu et al. (1995) found that the level of IL-1α paralleled the extent of DNA cross-linking after SM treatment and was accordingly a useful measure of cytotoxicity.

4.3. Consequences of TNFα production

Among the cytokines associated with the acute phase response, TNFα probably has the most diverse effects, and their consequences are especially pertinent to SM-induced cytotoxicity (Figure 8).

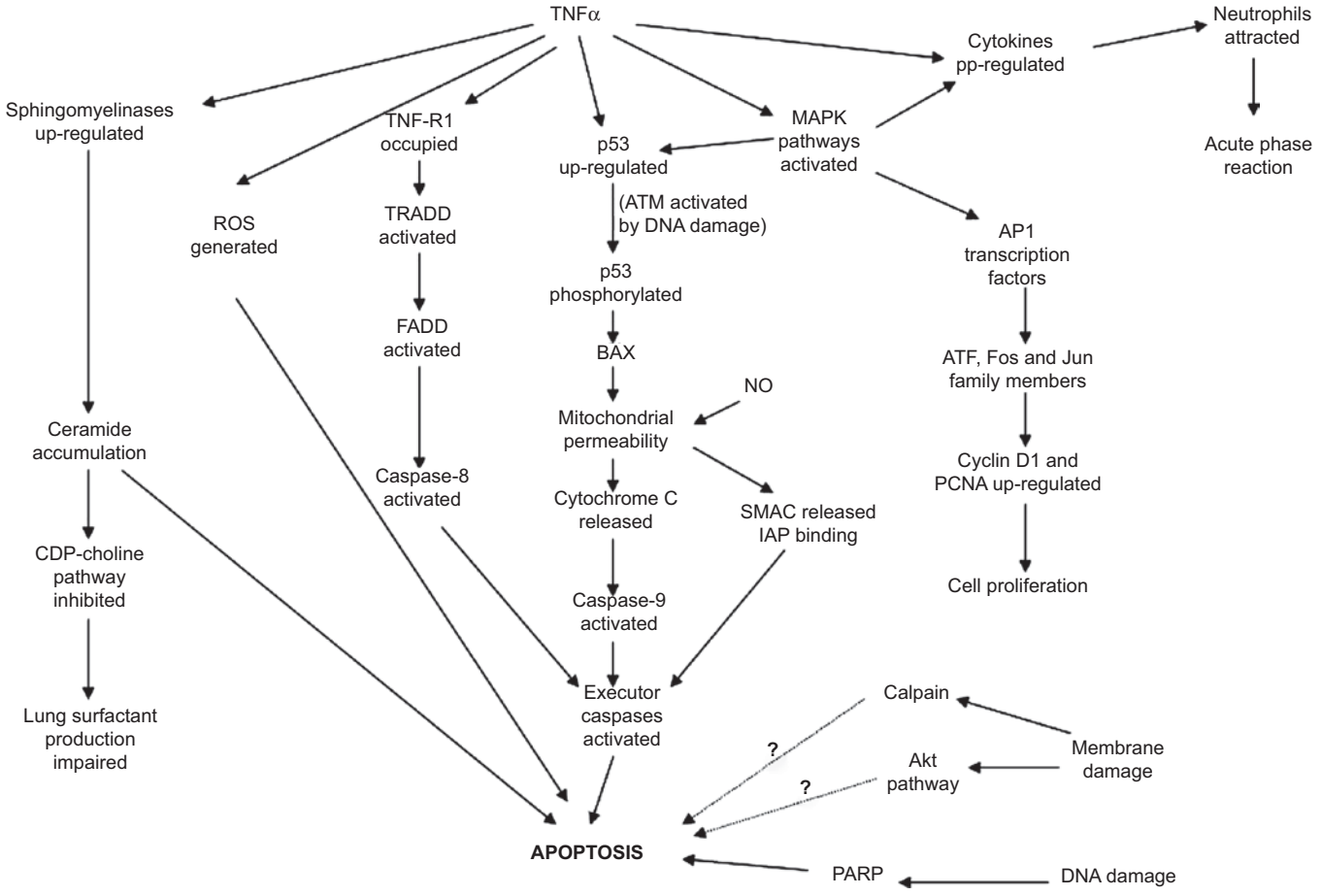


Figure 8. Involvement of TNFα in cellular responses to SM poisoning. TNFα, secreted by macrophages and other cell types, stimulates a range of different intracellular pathways, some of which promote the inflammatory response, while whereas others lead inter alia to apoptosis. In lung tissue, apoptosis, inflammation, and the down-regulation of lung surfactant production seem to be largely responsible for the pathological effects of SM (COPD, pulmonary edema, impaired gas exchange, etc.). In the skin, apoptosis, inflammation, and disruption of the cytoskeleton and extracellular keratin structures (not shown in the diagram) seem to be largely responsible for the vesicant action of SM.

4.3.1. MAPK family activation

A major effect of TNFα in many cell types is activation of members of the MAPK family. Exposure of pulmonary epithelial cells to CEES engages all members of the family (extracellular signal-regulated kinase 1/2 [ERK1/2], p38, and JNK1/2) within 1 hour, thus activating activator protein 1 (AP-1) transcription factors and increasing the protein levels of Fos, which in turn affects activating transcription factor (ATF) and Jun family members (Rebholz et al., 2008). Apart from cytokine production (see above), the consequences of MAPK activation include the up-regulation of cyclin D1 and the cell differentiation marker proliferating cell nuclear antigen (PCNA).

Along with the direct activation of c-*fos*, these events tend to promote cell proliferation. On the face of it, this conflicts with the major antiproliferative responses to SM exposure discussed below. In lung epithelia, enhanced proliferation may be an effective defensive reaction or a contributor to the lung injury (Mukhopadhyay et al., 2008); however, the latter seems more likely, since abnormal epithelial growth and cellular infiltration, together with continuous lung inflammation, are injurious (Allon et al., 2009). Control of AP-1 signaling may also mediate the protective effect of antioxidant liposomes against CEES-induced lung injury, according to evidence from animal experiments (Mukhopadhyay et al., 2009).

4.3.2. Sphingomyelinase activation

Intratracheal CEES in guinea pigs caused massive local hemorrhaging and edema into the alveoli, as in rats, and TNFα levels were markedly elevated (Chatterjee et al., 2003; Das et al., 2003). TNFα activated sphingomyelinases and hence caused a persistent local accumulation of ceramides, which promote apoptosis.

Since a major component of lung surfactant is dipalmitoyl phosphatidylcholine and the major pathway for its synthesis is the cytidine diphosphocholine (CDP-choline) pathway, inhibition of that pathway by ceramides (cf. Chatterjee et al., 2003; Das et al., 2003) might explain the underproduction of surfactant after SM inhalation. Indeed, dose-dependent inhibition of choline phosphotransferase by ceramides was observed after intubation of guinea pig lungs with CEES (Sinha Roy et al., 2005).

4.3.3. Nitric oxide

Several in vitro and experimental studies have shown that nitric oxide (NO) may play an important role in the acute or chronic phase of mustard agent toxicity (Gao et al., 2008; Yaren et al., 2007; Ishida et al., 2008; Ghazanfari et al., 2009a). Among the genes up-regulated in response to TNFα stimulation in some cell types is inducible nitric oxide synthetase (iNOS) (Ruimi et al., 2010). This probably contributes to SM-induced cytotoxicity; for example, acute epidermal inflammation is reduced when iNOS expression is inhibited, e.g., by iodine treatment (Nyska et al., 2001). In pulmonary epithelial cells and perhaps other cell types, SM causes concentration- and time-dependent production of iNOS, presumably via TNFα; it also activates endothelial NOS (eNOS) following translocation from the plasma membrane, possibly a consequence of direct membrane damage (Steinritz et al., 2009).

In vitro, exposure to SM substantially enhances expression of inducible nitric oxide synthase (iNOS) and intracellular NO production in human tracheobronchial and small airway epithelial cells (Gao et al., 2007, 2008). Increased iNOS activity is associated with enhanced lipid peroxidation and inflammatory cell infiltration in a rat model of lung SM toxicity (Yaren et al., 2007).

Notably, the effects of mustard agents may vary depending on the type of exposed cell/tissue. In vitro, SM suppresses iNOS expression in cultured normal human epidermal keratinocytes by ~50%, and this effect may explain the delayed healing of SM-induced skin wounds (Ishida et al., 2008). On the other hand, a study on Sardasht victims of mustard agent revealed elevated levels of serum nitric oxide among patients with late dermatological complications (Ghazanfari et al., 2009b). Serum nitric oxide concentrations increased with increasing severity of dermatological sequelae in the exposed patients (Ghazanfari et al., 2009b). However, the latter group (Ghazanfari et al., 2009a) reported no association between the serum NO levels and pulmonary problems, as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) classification, in the victims (Ghazanfari et al., 2009a). Instead, they found higher serum levels of NO among patients complaining of excess sputum production (Ghazanfari et al., 2009a). Exhaled NO was measured in a group of Iranian victims with chronic respiratory symptoms; this was found to be lower in steroid-off patients with obstructive lung disease than in healthy nonsmokers (unpublished data).

4.3.4. p53

Transcription of the pro-apoptotic protein p53 is strongly promoted by TNFα via the ERK/JNK pathways. Quantitative studies show that there is clear stress-dose-response effect on p53 protein levels, the stress being DNA damage (Nishizuka et al., 2008). The factor ARF (alternative reading frame) is also up-regulated by TNFα-activated MAPK pathways and stabilizes p53 (Zhang et al., 1998). The factors participating in the DNA damage response network, especially ATM (see above), phosphorylate p53. Phosphorylated p53 promotes DNA repair, is a major contributor to cell cycle arrest, and initiates one pathway of apoptosis. Some of its actions on gene transcription are modulated by Snai-2 (Pérez-Caro et al., 2008).

Activation of p53 has two major outcomes: cell cycle arrest or apoptosis. Its role in tissue responses to SM exposure may therefore be crucial. SM causes accumulation of p53 in cultured human keratinocytes (Rosenthal et al., 1998), and p53 mutations were found in the lung cancers in former workers in SM manufacturing plants (Manning et al., 1981; Easton et al., 1988; Nishimoto et al., 1987, Hosseini-Khalili et al., 2009). After SM-induced DNA damage to the central nervous system (CNS) in mice, neurobehavioral changes were associated with

increased p53 expression and therefore probably the mitochondrial apoptosis pathway (Sharma et al., 2009).

Several methods including DNA microarray technology have been used to characterize alterations in gene expression and consequent perturbation of molecular pathways in response to SM, many of them mediated by TNFα (Smith et al., 1993; Schlager and Hart, 2000; Dillman et al., 2004; Zhang et al., 2002; Platteborze, 2003; Rogers et al., 2004; Sabourin et al., 2004). SM causes a dose-dependent up-regulation of many p53-responsive genes in keratinocytes (Dillman et al., 2005), but it is not yet clear how many other genes are up-regulated independently of p53.

4.4. Cell death processes: Apoptosis and necrosis

SM causes both necrotic and apoptotic cell death in endothelia (Dabrowska et al., 1996; Atkins et al., 2000).

4.4.1. TNFα-associated apoptosis pathways

The foregoing discussion has indicated several possible contributors to the induction of apoptosis in SM-affected cells:

- Calpain activation resulting from membrane damage.
- PARP activation resulting from DNA damage.
- Ceramide production resulting from TNFα-induced activation of sphingomyelinases.

However, the two most generally important mechanisms of apoptosis caused by SM exposure are the extrinsic (death receptor, Fas) and intrinsic (mitochondrial) pathways, both related to TNFα. Kehe et al. (2009a) reviewed evidence linking SM-induced DNA damage to both pathways, details of which are well known. Binding of TNFα to the receptor TNF-R1 is instrumental in up-regulating inflammatory mediator genes but also activates the TNF-RSF1A–associated via death domain (TRADD), leading to activation of the Fas-associated death domain (FADD) and hence of caspase-8. Caspase-8 then activates the executor caspases and apoptosis proceeds. Independently of this pathway, phosphorylated p53 causes a Bax-related increase in mitochondrial membrane permeability; nitric oxide, produced by eNOS and the TNFα-up-regulated iNOS, has a similar effect. The release of SMAC (second mitochondria-derived activator of caspases) from the mitochondrion and subsequent binding to IAP (inhibitor of apoptosis) leads to caspase release. Concomitantly, the release of cytochrome *c* activates caspase-9, and again the executor caspases are activated.

4.4.2. Relationship of apoptosis to pathological effects of SM

Epithelial cell apoptosis may explain the significant lesions that appear in the respiratory tract during the months following SM exposure (Chatterjee et al., 2003; Das et al., 2003). It might also explain the effects of high SM dosage on the gastrointestinal tract (Graef et al., 1948; Papirmeister et al., 1991; Schonwald, 1992).

Using an in vitro cultured model of the human alveolar-capillary boundary, Emmler et al. (2007) found a marked time- and dose-dependent reduction of transbilayer electrical resistance associated with structural loss of both cell layers. This effect, which presumably contributes to pulmonary edema, was attributable to apoptosis, since markers including cytochrome *c*, p53, FADD, and procaspase-3 were significantly induced; IL-6 and IL-8 were also up-regulated (Pohl et al., 2009). SM-related cytotoxicity of the endothelial cells was indicated by adenylate kinase (AK) release and by TUNEL (terminal deoxynucleotidyl transferase dUTP *nick end labeling)* and Hoechst staining of the nuclei (Emmler et al., 2007).

Steinritz et al. (2007) also found evidence for apoptosis in cultured pulmonary A549 cells that was resistant to broad-spectrum caspase inhibitors. Apoptosis induced by SM appeared to be associated with a dominant caspase-8–mediated pathway, but bronchial epithelial cells were much more sensitive to SM than small airway epithelial cells; caspase-9 was activated only in the former, suggesting a mitochondrial pathway of apoptosis in the latter (Ray et al., 2008). Martin et al. (2009) reported similar results for immature mouse cortical neurons.

Mol et al. (2009) used specific inhibitors of caspase-8 and caspase-9 to show that SM-induced apoptosis is also initiated by both the death receptor and mitochondrial pathways in keratinocytes. Cells were morphologically better conserved when caspase-8 rather than caspase-9 activity was blocked. These authors further showed that (a) transmembrane enzymes of the "A disintegrin and metalloproteinase" family and (b) membrane-type metalloproteinases are implicated in the epidermal-dermal separation induced by SM. Also, TNFα-converting enzyme is involved in degrading cell-matrix adhesions, attenuating the response to epithelial-derived growth factor (EDGF) and releasing TNFα, a previously unsuspected mechanism contributing to the toxic effects of SM.

4.4.3. Necrosis

Mild dermal and epidermal necrosis is associated with SM-induced skin lesions (Momeni et al., 1992) and this is concurrent with the inflammatory response. Epidermal and follicular necrosis begins within a few hours of SM challenge to hairless guinea pig skin, accompanied by intracellular edema (Yourick et al., 1993). In the skin, melanocytes are more susceptible to necrotic death than keratinocytes. The DNA repair cofactor PCNA is present at much higher levels in resistant cell lines (Smith et al., 2001).

Tu et al. (2009) reported that p53 induces cathepsin Q, which cooperates with reactive oxygen species (ROS) to execute necrosis. The authors termed this mechanism "programmed necrotic death."

4.5. Involvement of reactive oxygen species and nuclear factor kappa B

Reactive oxygen species (ROS) are intimately associated with TNFα-induced apoptosis (Bubici et al., 2006), promoting cytochrome *c* release from mitochondria. ROS also have a range of other cytotoxic effects (e.g., oxidation of DNA and of GSH) that contribute to necrosis and cytolysis as well as the

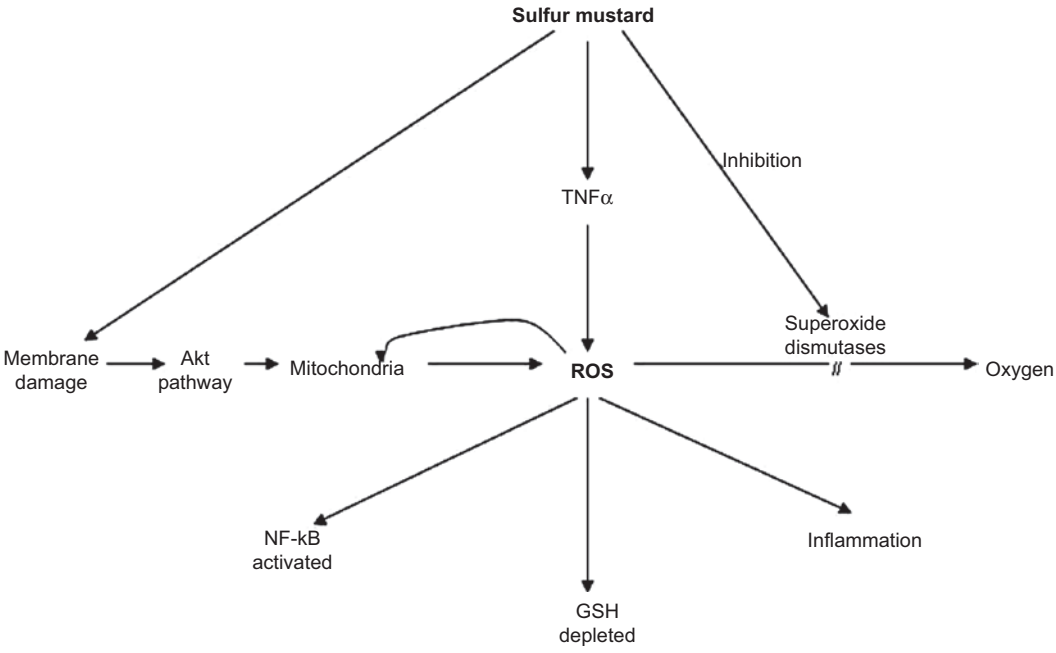


Figure 9. Involvement of reactive oxygen species in cellular responses to SM poisoning. ROS production as a result of TNFα stimulation was indicated in Fig.Figure 8, but ROS appear to mediate many of the cytotoxic actions of SM; see text for details.

inflammatory response. Mustard agents have been shown to increase ROS levels in affected tissues (Figure 9).

CEES causes mitochondrial dysfunction in airway epithelial cells within 4 hours of exposure and within 12 hours there is a marked increase in mitochondria-associated ROS production (Gould et al., 2009). CEES treatment also lowers the total superoxide dismutase (SOD) activity in lungs by direct inactivation of SOD-3 (Mukhopadhyay et al., 2006). Patients with moderate to severe lung injury as a result of SM exposure have lower pulmonary epithelial SOD levels than healthy controls, although their catalase levels are higher (Shohrati et al., 2008a). Chronic SM exposure may also generate oxidative stress in the mouse brain, triggering cytochrome *c* release and caspase-3 activation and precipitating neuronal apoptosis, contributing to neurobehavioral impairment (Sharma et al., 2009).

Nuclear factor kappa B (NF-κB) is also up-regulated by SM in cultured cells (Atkins et al., 2000; Schlager and Hart, 2000) and by CEES in guinea pig lung (Chatterjee et al., 2003). In general, TNFα inhibits proliferation and promotes apoptosis and NF-κB has the opposite effects, but the detailed relationships among TNFα, ROS, and NF-κB are complicated and depend on cell type (Szołtysek et al., 2008). TNFα production is induced by ROS via NF-κB in some cells (Chandel et al., 2000); in others, NF-κB mediates TNFα production in response to SM (cf. Atkins et al., 2000). NF-κB can oppose TNFα-induced apoptosis by interfering with JNK pathway, attenuating ROS production (Bubici et al., 2006). However, in many cell types, for example human umbilical vein endothelial cells, TNFα induces ROS production, which then up-regulates NF-κB (Mukherjee et al., 2005) and this apparently mediates inflammation in lung epithelia (Babbar and Casero, 2006).

Minsavage and Dillman (2007) found that in cell lines showing the classical rapid activation of NF-κB by TNFα, bifunctional alkylating agents such as SM also cause a slower, nonclassical activation mediated by p53 and the p90 ribosomal S6 kinase (p90RSK). Rebholtz et al. (2008) found that SM induced the classical NF-κB pathway in keratinocytes, strictly dependent on the transactivating subunit RelA. Concomitantly with the activation of NF-κB, the Raf-1/MEK1/2/ERK1/2/MSK1, MKK3/6/p38/MSK1, and MKK4/7/JNK1/2 pathways were induced, though c-Jun was not phosphorylated. NF-κB mediates the injury to the alveolar-capillary boundary studied by Emmler et al. (2007) and is directly up-regulated by SM in keratinocytes (Atkins et al., 2000); *N*-acetylcysteine attenuates this up-regulation. The induction of epithelial necrosis in the respiratory tract also appears to involve NF-κB (Dacre and Goldman 1996; Das et al., 2003). In the activation of sphingomyelinases by TNFα, NF-κB was only transiently elevated, so its potentially anti-apoptotic effects were ephemeral (Chatterjee et al., 2003; Das et al., 2003).

4.6. Protein phosphorylation cascades induced by SM

SM leads to the phosphorylation of a myriad of proteins. Using stable isotope labeling with amino acids in cell culture (SILAC) and immobilized metal affinity chromatography (IMAC) methods, Everley and Dillman (2010) identified numerous phosphorylated proteins following DNA damage in SM-treated cells. Among these, the levels of phosphorylated LIG1, RFC1, SRRM2, SVIL, TP53BP1, BCLAF1, CDK2, DPF2, and ZMYND8 were higher than normal. Subsequently, these authors designed a de novo construction of SM-specific protein interaction networks. Similarly, proteomic analysis of DNA-protein cross-linking

(DPC) by the antitumor nitrogen mustard, mechlorethamine, identified many proteins including those involved in cell motility, transcriptional regulation, chromatin remodeling, DNA supercoiling, DNA replication, glycolysis, initiation of apoptosis, and ribosome biogenesis (Loeber et al., 2009).

5. Conclusions

Although our understanding of the molecular mechanisms underlying the pathogenic effects of SM exposure is incomplete, enough detail is now known to explain the major points. Because it is highly reactive and bifunctional, SM alkylates most biomolecules, causing extensive damage to membrane constituents, cellular and extracellular proteins, and especially DNA. Proteolysis (induced, e.g., by plasminogen activator) and perturbation of the cytoskeleton appear to account for the disruption of tissue structure in skin, lungs, and gastrointestinal tract. Cells are lysed because of membrane damage and ATP depletion resulting from the response to DNA damage. Together, these effects recruit phagocytes and promote an acute phase reaction, which accounts for the effects of SM on the eyes and its well-known vesicant properties. Mechanisms of apoptosis and necrosis induced by SM have been thoroughly investigated, and the involvement of TNFα, NF-κB, ROS, and p53 is now well understood. These details have helped to elucidate the pathogenesis of chronic lung injury, including edema, impairment of the alveolar-capillary boundary, and onset of chronic obstructive pulmonary disease (COPD).

These advances in knowledge suggest methods for intervening in SM-related pathogenesis, or possibly reversing some of its effects. The success of studies using *N*-acetylcysteine and antioxidant-bearing liposomes are illustrative. Further studies of the effects of SM and the molecular and cellular level are likely to lead to new clinically applicable findings.

Declaration of interest

The present review article was financially supported by a joint grant from the Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, and the Research Center of Chemical Injuries, Baqiyatallah University of Medical Sciences. The authors′ affiliations are as shown on the title page. The authors have sole responsibility for the writing and content of the paper.

References

Abe Y, Sugisaki K, Dannenberg AM Jr. (1996). Rabbit vascular endothelial adhesion molecules: ELAM-1 is most elevated in acute inflammation, whereas VCAM-1 and ICAM-1 predominate in chronic inflammation. J Leukoc Biol 60:692–703.

Allon N, Amir A, Manisterski E, Rabinovitz I, Dachir S, Kadar T. (2009). Inhalation exposure to sulfur mustard in the guinea pig model: Clinical, biochemical and histopathological characterization of respiratory injuries. Toxicol Appl Pharmacol 241:154–162.

Anderson DR, Holmes WW, Lee RB, Dalal SJ, Hurst CG, Maliner BI, Newmark J, Smith WJ. (2006). Sulfur mustard-induced neutropenia: Treatment with granulocyte colony-stimulating factor. Mil Med 171:448–453.

Arroyo CM, Schafer RJ, Kurt EM, Broomfield CA, Carmichael AJ. (1999). Response of normal human keratinocytes to sulfur mustard (HD): Cytokine release using a non-enzymatic detachment procedure. Hum Exp Toxicol 18:1–11.

Ashby J, Tinwell H, Callander RD, Clare N. (1991). Genetic activity of the human carcinogen sulfur mustard toward Salmonella and the mouse bone marrow. Mutation Res 257:307–312.

Ashmore MH, Nathanail CP. (2008). A critical evaluation of the implications for risk based land management of the environmental chemistry of Sulphur Mustard. Environ Int 34:1192–1203.

Atkins KB, Lodhi IJ, Hurley LL, Hinshaw DB. (2000). NN-acetylcysteine and endothelial cell injury by sulfur mustard. J Appl Toxicol 20(Suppl. 1):S125–S128.

Auerbach C. (1943). Chemically induced mutations as rearrangements. Drosophila. Information Service 17:48–50.

Auerbach C, Moser H. (1950). Production of mutations by monochloro-mustards. Nature 166:1019–1020.

Auerbach C, Robson JM. (1946). Chemical production of mutations. Nature 157:302.

Auerbach C, Robson JM. (1947). Production of mutations by chemical substances. Proc Roy Soc Edin B 62:271–283.

Babbar N, Casero RA Jr. (2006). Tumor necrosis factor-alpha increases reactive oxygen species by inducing spermine oxidase in human lung epithelial cells: A potential mechanism for inflammation-induced carcinogenesis. Cancer Res 66:11125–11130.

Ball CR, Roberts JJ. (1970). DNA repair after mustard gas alkylation by sensitive and resistant Yoshida sarcoma cells in vitro. Chem Biol Interact 2:321–329.

Ball CR, Roberts JJ. (1972). Estimation of interstrand DNA cross-linking resulting from mustard gas alkylation of HeLa cells. Chem Biol Interact 4:297–303.

Bartek J, Bartkova J, Lukas J. (2007). DNA damage signalling guards against activated oncogenes and tumour progression. Oncogene 26:7773–7779.

Beck NV, Carrick WA, Cooper DB, Muir B. (2001). Extraction of thiodiglycol from soil using pressurised liquid extraction. J Chromatogr A 907:221–227.

Beckman L, Nordenson I. (1986). Interaction between some common genotoxic agents. Hum Hered 36:397–401.

Beheshti J, Mark EJ, Akbaei HM, Aslani J, Ghanei M. (2006). Mustard lung secrets: Long term clinicopathological study following mustard gas exposure. Pathol Res Pract 202:739–744.

Berger MR, Habs M, Schmähl D. (1986). Long-term toxicology effects of prednimustine in comparison with chlorambucil, prednisolone, and chlorambucil plus prednisolone in Sprague-Dawley rats. Semin Oncol 13:8–13.

Bitomsky N, Hofmann TG. (2009). Apoptosis and autophagy: Regulation of apoptosis by DNA damage signalling - —Roles of p53, p73 and HIPK2. FEBS J 276:6074–6083.

Black AT, Joseph LB, Casillas RP, Heck DE, Gerecke DR, Sinko PJ, Laskin DL, Laskin JD. (2010). Role of MAP kinases in regulating expression of antioxidants and inflammatory mediators in mouse keratinocytes following exposure to the half mustard, 2-chloroethyl ethyl sulfide. Toxicol Appl Pharmacol 245:352–360.

Black RM, Brewster K, Clarke RJ, Hambrook JL, Harrison JM, Howells DJ. (1992). Biological fate of sulphur mustard, 1,1′-thiobis(2-chloroethane): Isolation and identification of urinary metabolites following intraperitoneal administration to rat. Xenobiotica 22:405–418.

Blaha M, Bowers W Jr, Kohl J, DuBose D, Walker J, Alkhyyat A, Wong G. (2000). Effects of CEES on inflammatory mediators, heat shock protein 70A, histology and ultrastructure in two skin models. J Appl Toxicol 20(Suppl. 1):S101–S108.

Blayney DW, Longo DL, Young RC, Greene MH, Hubbard SM, Postal MC, Duffey PL, DeVita VT Jr. (1987). Decreasing risk of leukemia with prolonged followup after chemotherapy and radiotherapy for Hodgkin′s Hodgkin′s disease. N Engl J Med 316:710–714.

Boursnell JC, Cohen JA, Dixon M, Francis GE, Greville GD, Needham DM, Wormall A. (1946). Studies on mustard gas (β,βß,ß-dichlorodiethylsulphide) and some related compounds. 5. The fate of injected mustard gas (containing radioactive sulphur) in the animal body. Biochem J 40:756–764.

Boyland E, Horning ES. (1949). The induction of tumours with nitrogen mustards. Br J Cancer 3:118–123.

Bubici C, Papa S, Pham CG, Zazzeroni F, Franzoso G. (2006). The NF-kappaB-mediated control of ROS and JNK signaling. Histol Histopathol 21:69–80.

Byrne MP, Broomfield CA, Stites WE. (1996). Mustard gas crosslinking of proteins through preferential alkylation of cysteines. J Protein Chem 15:131–136.

Callaway S, Pearce K. (1958). Protection against systemic poisoning by mustard gas, di(2-chloroethyl)sulphide, by sodium thiosulphate and thiocit in the albino rat. Br J Pharmacol 13:395–398.

Capizzi RL, Papirmeister B, Mullins JM, Cheng E. (1974). The detection of chemical mutagens using the L5178y-Asn-murine leukemia in vitro and in a host-mediated assay. Cancer Res 34:3073–3082.

Cerny LC, Cerny ER. (1997). The effect of biological media on the hydrolysis of mustard simulants. Biomed Sci Instrum 33:535–540.

Chandel NS, Trzyna WC, McClintock DS, Schumacker PT. (2000). Role of oxidants in NF-kappa B activation and TNF-alpha gene transcription induced by hypoxia and endotoxin. J Immunol 165:1013–1021.

Chatterjee D, Mukherjee S, Smith MG, Das SK. (2003). Signal transduction events in lung injury induced by 2-chloroethyl ethyl sulfide, a mustard analog. J Biochem Mol Toxicol 17:114–121.

Cliby WA, Roberts CJ, Cimprich KA, Stringer CM, Lamb JR, Schreiber SL, Friend SH. (1998). Overexpression of a kinase-inactive ATR protein causes sensitivity to DNA-damaging agents and defects in cell cycle checkpoints. EMBO J 17:159–169.

Colvin M, Chabner BA. (1990). Alkylating agents. In: Chabner BA, Collins JM, eds. Cancer Chemotherapy: Principles and Practice. Philadelphia: JB Lippincott, 276–313.

Cowan FM, Yourick JJ, Hurst CG, Broomfield CA, Smith WJ. (1993). Sulfur mustard-increased proteolysis following in vitro and in vivo exposures. Cell Biol Toxicol 9:269–277.

Dabrowska MI, Becks LL, Lelli JL Jr, Levee MG, Hinshaw DB. (1996). Sulfur mustard induces apoptosis and necrosis in endothelial cells. Toxicol Appl Pharmacol 141:568–583.

Dacre JC, Goldman M. (1996). Toxicology and pharmacology of the chemical warfare agent sulfur mustard. Pharmacol Rev 48:289–326.

Das SK, Mukherjee S, Smith MG, Chatterjee D. (2003). Prophylactic protection by *N*-acetylcysteine against the pulmonary injury induced by 2-chloroethyl ethyl sulfide, a mustard analogue. J Biochem Mol Toxicol 17:177–184.

Davison C, Rozman RS, Smith PK. (1961). Metabolism of bis-bβ-chloroethyl sulfide (sulfur mustard gas). Biochem Pharmacol 7:65–74.

Detheux M, Jijakli H, Lison D. (1997). Effect of sulphur mustard on the expression of urokinase in cultured 3T3 fibroblasts. Arch Toxicol 71:243–249.

Devereaux A, Amundson DE, Parrish JS, Lazarus AA. (2002). Vesicants and nerve agents in chemical warfare: Decontamination and treatment strategies for a changed world. Postgrad Med 1:90–96.

Dillman JF 3rd, McGary KL, Schlager JJ. (2003). Sulfur mustard induces the formation of keratin aggregates in human epidermal keratinocytes. Toxicol Appl Pharmacol 193:228–236.

Dillman JF 3rd, McGary KL, Schlager JJ. (2004). An inhibitor of p38 MAP kinase downregulates cytokine release induced by sulfur mustard exposure in human epidermal keratinocytes. Toxicol In Vitro 18:593–599.

Dillman JF 3rd, Phillips CS, Dorsch LM, Croxton MD, Hege AI, Sylvester AJ, Moran TS, Sciuto AM. (2005). Genomic analysis of rodent pulmonary tissue following bis-(2-chloroethyl) sulfide exposure. Chem Res Toxicol 18:28–34.

Dillman JF 3rd, Hege AI, Phillips CS, Orzolek LD, Sylvester AJ, Bossone C, Henemyre-Harris C, Kiser RC, Choi YW, Schlager JJ, Sabourin CL. (2006). Microarray analysis of mouse ear tissue exposed to bis-(2-chloroethyl) sulfide: Gene expression profiles correlate with treatment efficacy and an established clinical endpoint. J Pharm Exp Ther 317:76–87.

Drasch G, Kretschmer E, Kauert G, von Meyer M. (1987). Concentrations of mustard gas [Bis(2-chloroethyl)sulfide] in the tissues of a victim of a vesicant exposure. J Forensic Sci 32:1788–1793.

Duchovic RJ, Vilensky JA. (2007). Mustard gas: Its pre-World War I history. J Chem Educ 84:944–948.

Easton D, Peto J, Doll R. (1988). Cancers of the respiratory tract in mustard gas workers. Br J Ind Med 45:652–659.

Einhorn N. (1978). Acute leukemia after chemotherapy. Cancer 41:444.

Emmler J, Hermanns MI, Steinritz D, Kreppel H, Kirkpatrick CJ, Bloch W, Szinicz L, Kehe K. (2007). Assessment of alterations in barrier functionality and induction of proinflammatory and cytotoxic effects after sulfur mustard exposure of an in vitro coculture model of the human alveolo-capillary barrier. Inhal Toxicol 19:657–665.

Enoch T, Norbury C. (1995). Cellular responses to DNA damage: Cell-cycle checkpoints, apoptosis and the roles of p53 and ATM. Trends. Biochem Sci 20:426–430.

Epstein J. (1984). Nitrogen mustard (mechlorethamine) and UVB photocarcinogenesis: A dose-response effect. J Invest Dermatol 83:320–322.

Everley PA, Dillman JF 3rd. (2010). A large-scale quantitative proteomic approach to identifying sulfur mustard-induced protein phosphorylation cascades. Chem Res Toxicol 23:20–25.

Evison D, Hinsley D, Rice P. (2002). Chemical weapons. BMJ 324:332–335.

Fahmy OG, Fahmy MJ. (1972). Mutagenic selectivity for the RNA-forming genes in relation to the carcinogenicity of alkylating agents and polycyclic aromatics. Cancer Res 32:550–557.

Fasth A, Sorbo B. (1973). Protective effect of thiosulphate and metabolic thiosulphate precursors against toxicity of nitrogen mustard (HN2). Biochem Pharmacol 22:1337–1351.

Feakes D. (2003). Global society and biological and chemical weapons. In: Kaldor M, Anheier H, Glasius M, eds. Global Civil Society Yearbook. Oxford: Oxford University Press, 87–117.

Fisher B, Rockette H, Fisher ER, Wickerham DL, Redmond C, Brown A. (1985). Leukemia in breast cancer patients following adjuvant chemotherapy or postoperative radiation: The NSABP experience. J Clin Oncol 3:1640–1658.

Fox BW, Fox M. (1973). The nature of the resistance to methylene dimethane sulphonate in the Yoshida sarcoma. Br J Cancer 28:81.

Francis GE, Richards DE, Wormall A. (1957). The mechanism of the reaction between di-(2-chloroethyl) sulphone (mustard-gas sulphone) and amino acids. Biochem J 66:142–144.

Gao X, Ray R, Xiao Y, Barker PE, Ray P. (2007). Inhibition of sulfur mustard-induced cytotoxicity and inflammation by the macrolide antibiotic roxithromycin in human respiratory epithelial cells. BMC Cell Biol 8:17.

Gao X, Ray R, Xiao Y, Ray P. (2008). Suppression of inducible nitric oxide synthase expression and nitric oxide production by macrolide antibiotics in sulfur mustard-exposed airway epithelial cells. Basic Clin Pharmacol Toxicol 103:255–261.

Ghabili K, Agutter PS, Ghanei M, Ansarin K, Shoja MM. (2010). Mustard gas toxicity: The acute and chronic pathological effects. J Appl Toxicol 30:627–643.

Ghanei M, Harandi AA. (2007). Long term consequences from exposure to sulfur mustard: A review. Inhal Toxicol 19:451–456.

Ghanei M, Tazelaar HD, Chilosi M, Harandi AA, Peyman M, Akbari HM, Shamsaei H, Bahadori M, Aslani J, Mohammadi A. (2008). An international collaborative pathologic study of surgical lung biopsies from mustard gas-exposed patients. Respir Med 102:825–830.

Ghazanfari T, Sharifnia Z, Yaraee R, Pourfarzam S, Kariminia A, Mahlojirad M, Faghihzadeh S, Jalali-Nodoushan MR, Ardestani SK, Soroush MR, Amiri S, Hassan ZM, Ghavami S, Ghanei M. (2009a). Serum soluble Fas ligand and nitric oxide in long-term pulmonary complications induced by sulfur mustard: Sardasht-Iran Cohort Study. Int Immunopharmacol 9:1489–1493.

Ghazanfari T, Yaraee R, KiaSalari Z, Hedayati F, Ghasemi H, Pourfarzam S, Soroush MR, Faghihzadeh S. (2009b). Evaluation of serum levels of nitric oxide in chemical victims of Sardsht 20 years after sulfure mustard exposure [Persian]. Iran J War Public Health 1:9–23.

Gilbert RM, Rowland S, Davison CL, Papirmeister B. (1975). Involvement of separate pathways in the repair of mutational and lethal lesions induced by a monofunctional sulfur mustard. Mutation Res 28:257–275.

Gilchrist HL. (1928). A Comparative Study of World War Casualties from Gas and Other Weapons. Washington, DC: U.S. Government Printing Office.

Gilman A, Philips FS. (1946). The biological actions and therapeutic applications of the bβ-chloroethyl amines and sulfides. Science 103:409–415.

Goodwin B. (1998). Keen As Mustard: Britain´s Britain´s Horrific Chemical Warfare Experiments in Australia. St. Lucia: University of Queensland Press.

Gould NS, White CW, Day BJ. (2009). A role for mitochondrial oxidative stress in sulfur mustard analog 2-chloroethyl ethyl sulfide-induced lung cell injury and antioxidant protection. J Pharmacol Exp Ther 328:732–739.

Graef I, Karnofsky DA, Jager VB, Krichesky B, Smith HW. (1948). The clinical and pathologic effects of the nitrogen and sulfur mustards in laboratory animals. Am J Pathol 24:1–47.

Gray PJ. (1989). A Literature Review on the Mechanism of Action of Sulphur and Nitrogen Mustard. Maribyrong, Australia: DSTO Materials Research Laboratory, Maribyrong. AD-A125-070.

Greene MH, Boice JD Jr, Greer BE, Blessing JA, Dembo AJ. (1982). Acute nonlymphocytic leukemia after therapy with alkylating agents for ovarian cancer. N Engl J Med 307:1416–1421.

Gross CL, Watson CV, Petrali J, Papirmeister B. (1981). Effect of sulfur mustards on lysosomes from rat liver in vitro. Toxicol Appl Pharmacol 61:147–151.

Gross CL, Meier HL, Papirmeister B, Brinkley FB, Johnson JB. (1985). Sulfur mustard lowers nicotinamide adenine dinucleotide concentrations in human skin grafted to athymic nude mice. Toxicol Appl Pharmacol 81:85–90.

Gross CL, Innace JK, Hovatter RC, Meier HL, Smith WJ. (1993). Biochemical manipulation of intracellular glutathione levels influences cytotoxicity to isolated human lymphocytes by sulfur mustard. Cell Biol Toxicol 9:259–267.

Guthrie F. (1859). On some derivatives of the olefines. Quart J Chem Soc 12:109–126.
Guthrie F. (1860). On some derivatives of the olefines. Quart J Chem Soc 13:129–135.
Habraken Y, Ludlum D. (1989). Release of chloroethyl ethyl sulfide-modified DNA bases by bacterial 3-methyladenine-DNA glycosylases I and II. Carcinogenesis 10:489–492.
Haince JF, Kozlov S, Dawson VL, Dawson TM, Hendzel MJ, Lavin MF, Poirier GG. (2007). Ataxia telangiectasia mutated (ATM) signaling network is modulated by a novel poly(ADP-ribose)-dependent pathway in the early response to DNA-damaging agents. J Biol Chem 282:16441–16453.
Hambrook JL, Howells DJ, Schock C. (1993). Biological fate of sulphur mustard (1,1′-thiobis(2-chloroethane)): Uptake, distribution and retention of 35S in skin and in blood after cutaneous application of 35S-sulphur mustard in rat and comparison with human blood in vitro. Xenobiotica 23:537–561.
Han S, Espinoza LA, Liao H, Boulares AH, Smulson ME. (2004). Protection by antioxidants against toxicity and apoptosis induced by the sulphur mustard analog 2-chloroethylethyl sulphide (CEES) in Jurkat T cells and normal human lymphocytes. Br J Pharmacol 141:795–802.
Hosseini-Khalili A, Haines DD, Modirian E, Soroush M, Khateri S, Joshi R, Zendehdel K, Ghanei M, Giardina C. (2009). Mustard gas exposure and carcinogenesis of lung. Mutat Res 678:1–6.
Husain K, Dube SN, Sugendran K, Singh R, Das Gupta S, Somani SM. (1996). Effect of topically applied sulphur mustard on antioxidant enzymes in blood cells and body tissues of rats. J Appl Toxicol 16:245–248.
IARC. (1975). Some Aziridines, N-, S- and O-Mustards and Selenium. In: International Agency for Research on Cancer. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Lyon: IARC, 9:181–192.
Institute of Medicine. (1993). Veterans at Risk: The Health Effects of Mustard Gas and Lewisite. Washington, DC: National Academies Press.
Ishida H, Ray R, Ray P. (2008). Sulfur mustard downregulates iNOS expression to inhibit wound healing in a human keratinocyte model. J Dermatol Sci 49:207–216.
Ivarsson U, Nilsson H, Santesson J. (1992). A FOA Briefing Book on Chemical Weapons: Threat, Effects, and Protection. Umeå: National Defence Research Establishment.
Jackson KE. (1936). The history of mustard gas. J Tennessee Acad Sci 11:98–106.
Jacques J. (1991). First victims of Yperite. New J Chem 15:3–4.
Jafari M. (2007). Dose- and time-dependent effects of sulfur mustard on antioxidant system in liver and brain of rat. Toxicology 231:30–39.
Jensen KA, Kirk I, Westergaard M. (1950). Mutagenic activity of some mustard gas compounds. Nature 166:1020–1021.
Jones GB. (1998). From mustard gas to medicines: The history of modern cancer chemotherapy. Chem Herit 15:8–9, 40–42.
Jowsey PA, Williams FM, Blain PG. (2009). DNA damage, signalling and repair after exposure of cells to the sulphur mustard analogue 2-chloroethyl ethyl sulphide. Toxicology 257:105–112.
Kaina B. (1998). Critical steps in alkylation-induced aberration formation. Mutat Res 404:119–124.
Kehe K, Szinicz L. (2005). Medical aspects of sulphur mustard poisoning. Toxicology 214:198–209.
Kehe K, Balszuweit F, Emmler J, Kreppel H, Jochum M, Thiermann H. (2008). Sulfur mustard research-strategies for the development of improved medical therapy. Eplasty 8:e32.
Kehe K, Balszuweit F, Steinritz D, Thiermann H. (2009a). Molecular toxicology of sulfur mustard-induced cutaneous inflammation and blistering. Toxicology 263:12–19.
Kehe K, Thiermann H, Balszuweit F, Eyer F, Steinritz D, Zilker T. (2009b). Acute effects of sulfur mustard injury--—Munich experiences. Toxicology 263:3–8.
Khateri S, Wangerin R. (2008). Denied Truths. Tehran: The Center for Women and Family Affairs.
Kohn KW, Steibigel NH, Spears CL. (1965). Cross-linking and repair of DNA in sensitive and resistant strains of E. coli treated with nitrogen mustard. Proc Natl Acad Sci U S A 53:1154–1160.
Koletsky AJ, Bertino JR, Farber LR, Prosnitz LR, Kapp DS, Fischer D, Portlock CS. (1986). Second neoplasms in patients with Hodgkin′s disease following combined modality therapy: The Yale experience. J Clin Oncol 4:311–317.
Lakshmana Rao PV, Vijayaraghavan R, Bhaskar AS. (1999). Sulphur mustard induced DNA damage in mice after dermal and inhalation exposure. Toxicology 139:39–51.
Langford AM, Hobbs MJ, Upshall DG, Blain PG, Williams FM. (1996). The effect of sulphur mustard on glutathione levels in rat lung slices and the influence of treatment with arylthiols and cysteine esters. Hum Exp Toxicol 15:619–624.
Lardot C, Dubois V, Lison D. (1999). Sulfur mustard upregulates the expression of interleukin-8 in cultured human keratinocytes. Toxicol Lett 110:29–33.
Lawley PD, Brookes P. (1965). Molecular mechanism of the cytotoxic action of difunctional alkylating agents and of resistance to this action. Nature 206:480–483.
Lawley PD, Brookes P. (1968). Cytotoxicity of alkylating agents towards sensitive and resistant strains of *Escherichia coli* in relation to extent and mode of alkylation of cellular macromolecules and repair of alkylation lesions in deoxyribonucleic acids. Biochem J 109:433–447.
Lee WR. (1975). Comparison of the mutagenic effects of chemicals and ionizing radiation using Drosophila melanogaster test systems. In: Nygaard OF, Adler HI, Sinclair WK, eds. Radiation Research: Biomedical, Chemical, and Physical Perspectives. New York: Academic Press, 976–983.
Liu RN. (2008). Oxidative stress, plasminogen activator inhibitor 1, and lung fibrosis. Antioxid Redox Signal 10:303–319.
Loeber RL, Michaelson-Richie ED, Codreanu SG, Liebler DC, Campbell CR, Tretyakova NY. (2009). Proteomic analysis of DNA-protein cross-linking by antitumor nitrogen mustards. Chem Res Toxicol 22:1151–1162.
Ludlum DB, Metha JR, Steiner RF, DeWitt J. (1978). Physical properties of poly(3,*N*-4-ethenocytidylic acid). Biophys Chem 7:339–346.
Ludlum DB, Tong WP, Mehta JR, Kirk MC, Papirmeister B. (1984). Formation of O6-ethylthioethyldeoxyguanosine from the reaction of chloroethyl ethyl sulfide with deoxyguanosine. Cancer Res 44:5698–5701.
Ludlum DB, Kent S, Mehta JR. (1986). Formation of O6-ethylthioethylguanine in DNA by reaction with the sulfur mustard, chloroethyl sulfide, and its apparent lack of repair by O6-alkylguanine DNA alkyltransferase. Carcinogenesis 7:1203–1206.
Luening KG. (1951). Mustard gas and gynandromorph production in *Drosophila melanogaster*. Hereditas 37:488–500.
Lyon A. (2008). Iran′s Chemical Ali survivors still bear scars. Reuters [Online]. Available at: http://www.reuters.com/article/topNews/idUSBLA84491620080709?feedType=RSS&feedName=topNews&rpc=22&sp=true. Accessed on 26 February 2010.
Manning KP, Skegg DC, Stell PM, Doll R. (1981). Cancer of the larynx and other occupational hazards of mustard gas workers. Clin Otolaryngol Allied Sci 6:165–170.
Marshall VC. (1987). Case histories of toxic releases. In: Marshall VC, ed. Major Chemical Hazards. Chichester: Ellis Horwood, 326–380.
Martin LJ, Liu Z, Pipino J, Chestnut B, Landek MA. (2009). Molecular regulation of DNA damage-induced apoptosis in neurons of cerebral cortex. Cereb Cortex 19:1273–1293.
Mauro F, Elkind MM. (1968). Differences in survival variations during the growth cycle of cultured Chinese hamster cells treated with sulfur mustard and x-rays. Cancer Res 28:1150–1155.
Medema J. (1986). Mustard gas: The science of H. Nucl Biol Chem Def Technol Int 1:66–71.
Medvedeva N, Polyak Y, Kuzikova I, Orlova O, Zharikov G. (2008). The effect of mustard gas on the biological activity of soil. Environ Res 106:289–295.
Meier HL, Gross CL, Papirmeister B. (1984). The use of human models for validating the biochemical mechanism of mustard-induced injury and for developing and evaluating therapeutic regimens to prevent mustard gas incapacitation. In: Proceedngs of the Army Science Conference. New York: West Point.
Meisenberg BR, Melaragno AJ, Monroy RL. (1993). Granulocyte colony stimulating factor (G-CSF) for mustard-induced bone marrow suppression. Mil Med 158:470–474.
Micaily B, Moser C, Vonderheid EC, Koprowski C, Lightfoot D, Markoe A, Brady L. (1990). The radiation therapy of early stage cutaneous T-cell lymphoma. Int J Radiat Oncol Biol Phys 18:1333–1339.
Minsavage GD, Dillman JF 3rd. (2007). Bifunctional alkylating agent-induced p53 and nonclassical nuclear factor kappaB responses and cell death are altered by caffeic acid phenethyl ester: A potential role for antioxidant/electrophilic response-element signaling. J Pharmacol Exp Ther 321:202–212.
Miskin R, Reich E. (1980). Plasminogen activator: Induction of synthesis by DNA damage. Cell 19:217–224.
Mol MA, van den Berg RM, Benschop HP. (2008). Proteomic assessment of sulfur mustard-induced protein adducts and other protein modifications in human epidermal keratinocytes. Toxicol Appl Pharmacol 230:97–108.
Mol MA, van den Berg RM, Benschop HP. (2009). Involvement of caspases and transmembrane metalloproteases in sulphur mustard-induced

microvesication in adult human skin in organ culture: Directions for therapy. Toxicology 258:39–46.

Momeni AZ, Enshaeih S, Meghdadi M, Amindjavaheri M. (1992). Skin manifestations of mustard gas. A clinical study of 535 patients exposed to mustard gas. Arch Dermatol 128:775–780.

Mukherjee TK, Mukhopadhyay S, Hoidal JR. (2005). The role of reactive oxygen species in TNFalpha-dependent expression of the receptor for advanced glycation end products in human umbilical vein endothelial cells. Biochim Biophys Acta 1744:213–223.

Mukhopadhyay S, Rajaratnam V, Mukherjee S, Smith M, Das SK. (2006). Modulation of the expression of superoxide dismutase gene in lung injury by 2-chloroethyl ethyl sulfide, a mustard analog. J Biochem Mol Toxicol 20:142–149.

Mukhopadhyay S, Mukherjee S, Smith M, Das SK. (2008). Activation of MAPK/AP-1 signaling pathway in lung injury induced by 2-chloroethyl ethyl sulfide, a mustard gas analog. Toxicol Lett 181:112–117.

Mukhopadhyay S, Mukherjee S, Stone WL, Smith M, Das SK. (2009). Role of MAPK/AP-1 signaling pathway in the protection of CEES-induced lung injury by antioxidant liposome. Toxicology 261:143–151.

Murnane JP, Byfield JE. (1981). Irreparable DNA cross-links and mammalian cell lethality with bifunctional alkylating agents. Chem Biol Interact 38:75–76.

Naghii MR. (2002). Sulfur mustard intoxication, oxidative stress, and antioxidants. Mil Med 167:573–575.

Nasrat GE. (1954). Some cytological observations on the delayed effect of mustard gas. Nature 174:968–969.

New York Academy of Sciences. (1969). Biological effects of alkylating agents. Ann N Y Acad Sci 163:589–1029.

Niemann A. (1860). Über die Einwirkung des braunen Chlorschwefels auf Elaylgas. Liebigs Ann Chem Pharmacie 113:288–292.

Nishimoto Y, Yamakido M, Ishioka S, Shigenobu T, Yukutake M. (1987). Epidemiological studies of lung cancer in Japanese mustard gas workers. Princess Takamatsu Symp 18:95–101.

Nishizuka S, Ramalingam S, Spurrier B, Washburn FL, Krishna R, Honkanen P, Young L, Tsutomu S, Steeg PS, Austin J. (2008). Quantitative protein network monitoring in response to DNA damage. J Proteome Res 7:803–808.

Nyska A, Lomnitski L, Maronpot R, Moomaw C, Brodsky B, Sintov A, Wormser U. (2001). Effects of iodine on inducible nitric oxide synthase and cyclooxygenase-2 expression in sulfur mustard-induced skin. Arch Toxicol 74:768–774.

O´Neill O´,Neill HC, White CW, Veress LA, Hendry-Hofer TB, Loader JE, Min E, Huang J, Rancourt RC, Day BJ. (2010). Treatment with the catalytic metalloporphyrin AEOL 10150 reduces inflammation and oxidative stress due to inhalation of the sulfur mustard analog 2-chloroethyl ethyl sulfide. Free Radic Biol Med 48:1188–1196.

Papirmeister B, Davison CL. (1964). Elimination of sulfur mustard-induced products from DNA of *Escherichia coli*. Biochem Biophys Res Commun 17:608–617.

Papirmeister B, Westling AW, Schroer J. (1969). Mustard: The Relevance of DNA Damage to the Development of the Skin Lesion. Maryland: Medical Research Laboratory, Edgewood Arsenal.

Papirmeister B, Dorsey JK, Davison CL, Gross CL. (1970). Sensitization of DNA to endonuclease by adenine alkylation and its biological significance. Fed Proc 29:726.

Papirmeister B, Gross CL, Petrali JP, Hixson CJ. (1984a). Pathology produced by sulfur mustard in human skin grafts on athymic nude mice. I. Gross and light microscopic changes. Cutan Ocul Toxicol 3:371–392.

Papirmeister B, Gross CL, Petrali JP, Meier HL. (1984b). Pathology produced by sulfur mustard in human skin grafts on athymic nude mice. II. Ultrastructural changes. Cutan Ocul Toxicol 3:393–408.

Papirmeister B, Gross CL, Meier HL, Petrali JP, Johnson JB. (1985). Molecular basis for mustard-induced vesication. Fundam Appl Toxicol 5:S134–S149

Papirmeister B, Feister AJ, Robinson SI, Ford RD. (1991). Medical Defense Against Mustard Gas: Toxic Mechanisms and Pharmacological Implications. Boca Ratob, FL: CRC Press.

Paromov V, Qui M, Yang H, Smith M, Stone WL. (2008). The influence of *N*-acetyl-l-cysteine on oxidative stress and nitric oxide synthesis in stimulated macrophages treated with a mustard gas analogue. BMC Cell Biol 9:33.

Paull TT, Gellert M. (1999). Nbs1 potentiates ATP-driven DNA unwinding and endonuclease cleavage by the Mre11/Rad50 complex. Genes Dev 13:1276–1288.

Pérez-Caro M, Bermejo-Rodríguez C, González-Herrero I, Sánchez-Beato M, Piris MA, Sánchez-García I. (2008). Transcriptomal profiling of the cellular response to DNA damage mediated by Slug (Snai2). Br J Cancer 98:480–488.

Plant JE, Roberts JJ. (1971). Extension of the pre-DNA synthetic phase of the cell cycle as a consequence of DNA alkylation in Chinese hamster cells: A possible mechanism of DNA repair. Chem Biol Interact 3:343–351.

Platteborze PL. (2003). Effects of sulfur mustard on transcription in human epidermal keratinocytes: Analysis by mRNA differential display. J Appl Toxicol 23:249–254.

Pohanka M, Stetina R. (2009). Shift of oxidants and antioxidants levels in rats as a reaction to exposure to sulfur mustard. J Appl Toxicol 29:643–647.

Pohl C, Papritz M, Moisch M, Wübbeke C, Hermanns MI, Uboldi C, Dei-Anang J, Mayer E, Kirkpatrick CJ, Kehe K. (2009). Acute morphological and toxicological effects in a human bronchial coculture model after sulfur mustard exposure. Toxicol Sci 112:482–489.

Price CC, Gaucher GM, Konero P, Shibakowa R, Sowa JR, Yamaguchi M. (1968). Relative reactivities for monofunctional nitrogen mustard alkylation of nucleic acid components. Acta Biochim Biophys Hungarica 166:327–359.

Pu Y, Lin P, Vaughan FL, Bernstein IA. (1995). Appearance of interleukin 1 alpha relates DNA interstrand cross-links and cytotoxicity in cultured human keratinocytes exposed to bis-(2-chloroethyl) sulfide. J Appl Toxicol 15:477–482.

Ray R, Keyser B, Benton B, Daher A, Simbulan-Rosenthal CM, Rosenthal DS. (2008). Sulfur mustard induces apoptosis in cultured normal human airway epithelial cells: Evidence of a dominant caspase-8-mediated pathway and differential cellular responses. Drug Chem Toxicol 31:137–148.

Rebholz B, Kehe K, Ruzicka T, Rupec RA. (2008). Role of NF-kappaB/RelA and MAPK pathways in keratinocytes in response to sulfur mustard. J Invest Dermatol 128:1626–1632.

Reid EE. (1958). Organic Chemistry of Bivalent Sulfur. New York: Chemical Publishing.

Reid BD, Walker IG. (1966). Resistance to sulfur mustard: A comparison of some properties of strain L cells and a resistant subline. Cancer Res 26:1801–1805.

Reid BD, Walker IG. (1969). The response of mammalian cells to alkylating agents. II. On the mechanism of the removal of sulfur mustard-induced cross links. Biochim Biophys Acta 179:179–188.

Renshaw B. (1946). Mechanisms in production of cutaneous injuries by sulphur and nitrogen mustards. In: Chemical Warfare Agents and Related Chemical Problems. Washington DC: National Defense Research Commmittee of the Office of Scientific Research and Development, 479–518.

Richardson GM. (1897). Obituary for Viktor Meyer. J Am Chem Soc 19:918–921.

Riches J, Read RW, Black RM. (2007). Analysis of the sulphur mustard metabolites thiodiglycol and thiodiglycol sulphoxide in urine using isotope-dilution gas chromatography-ion trap tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 845:114–120.

Ricketts KM, Santai CT, France JA, Graziosi AM, Doyel TD, Gazaway MY, Casillas RP. (2000). Inflammatory cytokine response in sulfur mustard-exposed mouse skin. J Appl Toxicol 20:S73–S76

Rikimaru T, Nakamura M, Yano T, Beck G, Habicht GS, Rennie LL, Widra M, Hirshman CA, Boulay MG, Spannhake EW, et al. (1991). Mediators, initiating the inflammatory response, released in organ culture by full-thickness human skin explants exposed to the irritant, sulfur mustard. J Investig Dermatol 96:888–897.

Roberts JJ, Warwick GP. (1963). Studies of the mode of action of alkylating agents. VI. The metabolism of bis-2-chloroethylsulphide (mustard gas) and related compounds. Biochem Pharmacol 12: 1329–1334.

Roberts JJ, Kotsaki-Kovatsi VP. (1986). Potentiation of sulfur mustard or cisplatin-induced toxicity by caffeine in Chinese hamster cells correlates with formation of DNA double-strand breaks during replication on a damaged template. Mutation Res 165:207–220.

Roberts JJ, Warwick GP. (1963). Studies of the mode of action of alkylating agents. VI. The metabolism of bis-2-chloroethylsulphide (mustard gas) and related compounds. Biochem Pharmacol 12:1329–1334.

Roberts JJ, Brent TP, Crathorn AR. (1968). Mechanism of the cytotoxic action of alkylating agents on mammalian cells. In: Campbell PN, ed. Interaction of Drugs and Subcellular Components in Animal Cells. Boston: Little, Brown, 5–27.

Roberts JJ, Brent TP, Crathorn AR. (1971). Evidence for the inactivation and repair of the mammalian DNA template after alkylation by mustard gas and half mustard gas. Eur J Cancer 7:515–524.

Roberts JJ, Friedlos F, Scott D, Ormerod MG, Rawlings CJ. (1986). The unique sensitivity of Walker rat tumour cells to difunctional agents is associated

with a failure to recover from inhibition of DNA synthesis and increased chromosome damage. Mutation Res 166:169–181.
Rogers JV, Choi YW, Kiser RC, Babin MC, Casillas RP, Schlager JJ, Sabourin CL. (2004). Microarray analysis of gene expression in murine skin exposed to sulfur mustard. J Biochem Mol Toxicol 18:289–299.
Rosenthal DS, Simbulan-Rosenthal CM, Iyer S, Spoonde A, Smith W, Ray R, Smulson ME. (1998). Sulfur mustard induces markers of terminal differentiation and apoptosis in keratinocytes via a Ca2+-calmodulin and caspase-dependent pathway. J Investig Dermatol 111: 64–71.
Ross WCJ. (1962). Biological Alkylating Agents: Fundamental Chemistry and Design of Compounds for Selective Toxicity. London: Butterworths.
Ruimi N, Petrova RD, Agbaria R, Sussan S, Wasser SP, Reznick AZ, Mahajna J. (2010). Inhibition of TNF#-induced iNOS expression in HSV-tk transduced 9L glioblastoma cell lines by Marasmius oreades substances through NF-#B- and MAPK-dependent mechanisms. Mol Biol Rep 37:3801–3812.
Sabourin CL, Petrali JP, Casillas RP. (2000). Alterations in inflammatory cytokine gene expression in sulfur mustard-exposed mouse skin. J Biochem Mol Toxicol 14:291–302.
Sabourin CL, Danne MM, Buxton KL, Casillas RP, Schlager JJ. (2002). Cytokine, chemokine and matrix metalloproteinase response after sulfur mustard injury to weanling pig skin. J Biochem Mol Toxicol 16:263–272.
Sabourin CL, Rogers JV, Choi YW, Kiser RC, Casillas RP, Babin MC, Schlager JJ. (2004). Time- and dose-dependent analysis of gene expression using microarrays in sulfur mustard-exposed mice. J Biochem Mol Toxicol 18:300–312.
Sasser LB, Cushing JA, Dacre JC. (1996). Two-generation reproduction study of sulfur mustard in rats. Reprod Toxicol 10:311–319.
Savage JR, Breckon G. (1981). Differential effects of sulfur mustard on S phase cells of primary fibroblast cultures from Syrian hamsters *Mesocricetus auratus*. Mutation Res 84:375–387.
Schardein JL. (1985). Cancer chemotherapeutic agents. In: Schardein JL. Chemically Induced Birth Defects. New York: Dekker, 467–520.
Schlager JJ, Hart BW. (2000). Stress gene activity in HepG2 cells after sulfur mustard exposure. J Appl Toxicol 20:395–405.
Schneider M, Poth EJ, Levin WC. (1948). Nitrogen mustard in the treatment of Hodgkin′s disease; a case report with discussion. Tex Rep Biol Med 6:117–122.
Schonwald S. (1992). Mustard gas. PSR Quarterly 2:92–97.
Scott D, Bigger TR. (1972). The induction of chromosomal aberrations by sulphur mustard in marsupial lymphocytes. Chromosomes Today 3:162–176.
Scott D, Fox M, Fox BW. (1974). The relationship between chromosomal aberrations, survival, and DNA repair in tumour cell lines of differential sensitivity to x-rays and sulphur mustard. Mutation Res 22:207–221.
Sharma M, Vijayaraghavan R, Ganesan K. (2008). Comparison of toxicity of selected mustard agents by percutaneous and subcutaneous routes. Indian J Exp Biol 46:822–830.
Sharma DR, Sunkaria A, Bal A, Bhutia YD, Vijayaraghavan R, Flora SJ, Gill KD. (2009). Neurobehavioral impairments, generation of oxidative stress and release of pro-apoptotic factors after chronic exposure to sulphur mustard in mouse brain. Toxicol Appl Pharmacol 240:208–218.
Shohrati M, Ghanei M, Shamspour N, Jafari M. (2008a). Activity and function in lung injuries due to sulphur mustard. Biomarkers 13:728–733.
Shohrati M, Ghanei M, Shamspour N, Jafari M, Aslani J, Faskhami-Norozi A. (2008b). Effect of long-term pulmonary complications due to sulfur mustard on activation of enzymes in antioxidant system [Persian]. Kowsar Med J 13:65–70.
Shohrati M, Ghanei M, Shamspour N, Jafari M, Khasmakhi MN. (2009). Extracellular superoxide dismutase activity in lung injuries due to sulfur mustard [Persian]. J Qazvin Uni Med Sci 12:5–11.
Sidell FR, Hurst CG. (1992). Clinical considerations in mustard gas poisoning. In: Somani SM, ed. Chemical Warfare Agents. San Diego: Academic Press.
Sinha Roy S, Mukherjee S, Kabir S, Rajaratnam V, Smith M, Das SK. (2005). Inhibition of cholinephosphotransferase activity in lung injury induced by 2-chloroethyl ethyl sulfide, a mustard analog. J Biochem Mol Toxicol 19:289–297.
Smith CN, Lindsay CD, Hambrook JL. (2001). An in vitro comparison of the cytotoxicity of sulphur mustard in melanoma and keratinocyte cell lines. Hum Exp Toxicol 20:483–490.
Smith WJ, Saunders KM, Ruddle SE, Gross CL. (1993). Cytometric analysis of DNA changes induced by sulfur mustard. J Toxicol Cutan Ocul Toxicol 12:337–343.
Sobels FH. (1962). Rates of forward and reverse mutation in Drosophila after exposure to mustard gas and x-rays. Genetica 33:31–44.
Sobels FH, Van Steenis H. (1957). Chemical induction of crossing-over in *Drosophila* males. Nature 179:29–31.
Somani SM, Babu SR. (1989). Toxicodynamics of sulfur mustard. Int J Clin Pharmacol Ther Toxicol 27:419–435.
Steinritz D, Emmler J, Hintz M, Worek F, Kreppel H, Szinicz L, Kehe K. (2007). Apoptosis in sulfur mustard treated A549 cell cultures. Life Sci 80:2199–2201.
Steinritz D, Elischer A, Balszuweit F, Gonder S, Heinrich A, Bloch W, Thiermann H, Kehe K. (2009). Sulphur mustard induces time- and concentration-dependent regulation of NO-synthesizing enzymes. Toxicol Lett 188:263–269.
Stevens CM, Mylroie A. (1950). Mutagenic activity of ββ-chloroalkyl amines and sulphides.Nature 166:1019.
Strieter RM, Kunkel SL. (1994). Acute lung injury: The role of cytokines in the elicitation of neutrophils. J Investig Med 42:640–651.
Szoltysek K, Pietranek K, Kalinowska-Herok M, Pietrowska M, Kimmel M, Widlak P. (2008). TNFalpha-induced activation of NFkappaB protects against UV-induced apoptosis specifically in p53-proficient cells. Acta Biochim Pol 55:741–748.
Tchounwou PB, Wilson BA, Ishaque AB, Schneider J. (2001). Transcriptional activation of stress genes and cytotoxicity in human liver carcinoma cells (HepG2) exposed to 2,4,6-trinitrotoluene, 2,4-dinitrotoluene, and 2,6-dinitrotoluene. Environ Toxicol 6:209–216.
Tsuruta J, Sugisaki K, Dannenberg AM, Yoshimura T, Abe Y, Mounts P. (1996). The cytokines NAP-1(IL-8), MCP-1, IL-1beta and GRO in rabbit inflammatory skin lesions produced by the chemical irritant sulfur mustard. Inflammation 20:293–318.
Tu HC, Ren D, Wang GX, Chen DY, Westergard TD, Kim H, Sasagawa S, Hsieh JJ, Cheng E. H. (2009). The p53-cathepsin axis cooperates with ROS to activate programmed necrotic death upon DNA damage. Proc Natl Acad Sci U S A 106:1093–1098.
Tucker MA, Coleman CN, Cox RS. (1988). Risk of second cancers after treatment for Hodgkin′s Hodgkin′s disease. N Eng J Med 318:76–81.
van der Schans GP, Noort D, Mars-Groenendijk RH, Fidder A, Chau LF, de Jong LP, Benschop HP. (2002). Immunochemical detection of sulfur mustard adducts with keratins in the stratum corneum of human skin. Chem Res Toxicol 15:21–25.
Vickery HB. (1975). Hans Thacher Clarke: A Biographical Memoir. Washington DC: National Academy of Sciences.
Vidaković M, Poznanović G, Bode J. (2005). DNA break repair: Refined rules of an already complicated game. Biochem Cell Biol 83:365–373.
Vijayaraghavan R, Kulkarni A, Pant SC, Kumar P, Rao PV, Gupta N, Gautam A, Ganesan K. (2005). Differential toxicity of sulfur mustard administered through percutaneous, subcutaneous, and oral routes. Toxicol Appl Pharmacol 202:180–188.
Vojvodic V, Milosavljevic Z, Boskovic B, Bojanic N. (1985). The protective effect of different drugs in rats poisoned by sulphur and nitrogen mustards. Fundam Appl Toxicol 5:S160–S168
Vycludic W. (1985). Detection of mustard gas bis (2-chloroethyl)-sulfide in urine. Foren Sci Int 28:131–136.
Walker IG. (1966). Sulfur mustard: Reaction with L-cells treated with 5-fluorodeoxyuridine. Science 151:99–101.
Walker IG, Reid BD. (1971). Some properties of substrains of L-cells with a decreased sensitivity to bis(2-chloroethyl)sulfide. Cancer Res 31:510–515.
Walker IG, Smith JF. (1969). Protection of L-cells by thiols and against the toxicity of sulfur mustard. Can J Physiol Pharmacol 47:143–151.
Ward JR, Seider RP. (1984). Activation energy for the hydrolysis of bis(2-chloroethyl) sulfide. Thermochimica Acta 81:343–348.
Warren JS, Yabroff KR, Mandel DM, Johnson KJ, Ward PA. (1990). Role of O2- – in neutrophil recruitment into sites of dermal and pulmonary vasculitis. Free Radic Biol Med 8:163–172.
Watson AP, Griffin GD. (1992). Toxicity of vesicant agents scheduled for destruction by the Chemical Stockpile Disposal Program. Environ Health Perspect 98:259–280.
Werrlein RJ, Madren-Whalley JS. (2000). Effects of sulfur mustard on the basal cell adhesion complex. J Appl Toxicol 1 (Suppl. 20):S115–S123.
West CJ. (1919). History of mustard gas. Science 49:412–417.
Wheeler GP. (1962). Studies related to the mechanisms of action of cytotoxic alkylating agents: A review. Cancer Res 22:651–688.
Wheeler GP. (1967). Some biochemical effects of alkylating agents. Proc Soc Exp Biol Med 26:885–892.
Wilde PE, Upshall DG. (1994). Cysteine esters protect cultured rodent lung slices from sulphur mustard. Hum Exp Toxicol 13:743–748.
Woessner JF Jr, Dannenberg AM Jr, Pula PJ, Selzer MG, Ruppert CL, Higuchi K, Kajiki A, Nakamura M, Dahms NM, Kerr JS, et al. (1990). Extracellular

collagenase, proteoglycanase and products of their activity, released in organ culture by intact dermal inflammatory lesions produced by sulfur mustard. J Invest Dermatol 95:717–726.

World Health Organization (1970). Health Aspects of Chemical and Biological Weapons: Report of a WHO Group of Consultants. WHO, Geneva.: WHO. Available at: http://www.who.int/csr/delibepidemics/en/TableofContents.pdf. Accessed on 30 May 2010.

Wormser U, Brodsky B, Proscura E, Foley JF, Jones T, Nyska A. (2005). Involvement of tumor necrosis factor-alpha in sulfur mustard-induced skin lesion; effect of topical iodine. Arch Toxicol 79:660–670.

Wulf HC, Aasted A, Darre E, Niebuhr E. (1985). Sister chromatid exchanges in fishermen exposed to leaking mustard gas shells ([letter).]. Lancet 1:690–691.

Yanagida J, Hozawa S, Ishioka S, Maeda H, Takahashi K, Oyama T, Takahashi M, Hakoda M, Akiyama M, Yamakido M. (1988). Somatic mutation in peripheral lymphocytes of former workers at the Okuno-jima poison gas factory. Jap J Cancer Res 79:1276–1283.

Yaren H, Mollaoglu H, Kurt B, Korkmaz A, Oter S, Topal T, Karayilanoglu T. (2007). Lung toxicity of nitrogen mustard may be mediated by nitric oxide and peroxynitrite in rats. Res Vet Sci 83:116–122.

Yourick JJ, Dawson JS, Benton CD, Craig ME, Mitcheltree LW. (1993). Pathogenesis of 2,2′-dichlorodiethyl sulfide in hairless guinea pigs. Toxicology 84:185–197.

Zhang P, Ng P, Caridha D, Leach RA, Asher LV, Novak MJ, Smith WJ, Zeichner SL, Chiang PK. (2002). Gene expressions in Jurkat cells poisoned by a sulphur mustard vesicant and the induction of apoptosis. Br J Pharmacol 137:245–252.

Zhang Y, Xiong Y, Yarbrough WG. (1998). ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell 92:725–734.

Zhang Z, Riviere JE, Monteiro-Riviere NA. (1995). Evaluation of protective effects of sodium thiosulfate, cysteine, niacinamide and indomethacin on sulfur mustard-treated isolated perfused porcine skin. Chem Biol Interact 96:249–262.