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RESEARCH ARTICLE

Sulphur Mustard Induced Toxicity, Mechanism of Action and Current Medical Management

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ABSTRACT

Sulphur mustard (SM), chemically, bis (2-chloroethyl) sulphide is a bifunctional alkylating agent that causes cutaneous blisters in human or animals. It was first used in the World War I. Since then, there have been 11 conflicts where SM allegedely had caused mass distruction. Additionally, discarded weapons and stockpiles periodically come to surface during agricultural or fishing activities leading to serious injury. Concerns for threat to modern societies by the serious effects of SM, agreements to ban its production and the use has been made as per 1993 chemical weapons convention (CWC) and agent destruction programs. This short review attempts to discuss the histroy, chemical nature, mechanism of toxicity, toxicokinetics, animal models used for SM induced skin and systemic lesions, pathogenesis of SM induced lesions including medical countermeasures for SM toxicity.

Keywords: Sulphur mustard, vesicants, blistering agents, animal model, therapeutics

1. INTRODUCTION

Earliest forms of chemical warfare agents (CWAs) were natural toxins from plants or animals, which were used to coat arrowheads, commonly referred to as 'arrow poisons'. These have been elaborately described in ancient Chinese literature. Successfully use of hellebores roots were also made in 600 BC by the Athenians to contaminate water supplies of besieged Greek city Cirrha. Spartan forces ignited pitch and sulphur to create toxic fumes during Peloponnesian War in 429 BC. With advancements in science and chemistry in the 19th century, the possibility of chemical warfare increased tremendously. The employment of chemicals in war has a long history[1,2]. World War I has been called the ''Chemist's War'' because it was the beginning of the modern era of chemical warfare. Chemical agents were used in wars, conflicts by terrorist and extremists, dictator's activities, malicious poisonings and executions. Most of the key chemical warfare agents used during the war included: chlorine in1774; hydrogen cyanide in1782; cyanogen chloride in 1802; phosgene in 1812; mustard agent in 1822; and chloropicrin in 1848[3].

During the studies of interactions between olefins and sulphur halogen compounds, mustard gas [bis [2-chloroethyl] sulphide] was first synthesized in 1822 by Despretz and in 1860 by Niemann and Guthrie[4]. Both investigators noted the typical vesicant properties of the agent. In 1886, V. Meyer was able to synthesize SM of higher purity.[5] In the distilled form; SM is designated as HD according to the US military code, which is used in the western countries. Other names include SM (typical odor), yperite (Ypres was the place of the first military use), Lost (acronym of the German chemists Lommel and Steinkopf who investigated the military use of the compound), and yellow cross [German shells were marked with a yellow cross skin damaging agent]. The United States and the British also researched mixing different chemicals with mustard agent. This work identified HL (a mixture of mustard agent and lewisite), HQ (sesqui mustard), the mixture of 40 per cent agent T and 60 per cent SM [SM] designated H agent T [bis-[2-[2-chloroethylthio] ethyl] ether is a similarly acting compound and HV (a thickened mustard agent). Both the sides also researched the nitrogen mustard agents[2].

SM is an effective vesicating [blistering] CW agent and since World War I, has been used in many conflicts which include its use by Italy against Ethiopia in 1936, by Japan against China in 1937, by Poland against Germany in 1939, by Egypt against Yemen from 1963 to 1967 and by Iraq against Iran in the 1980s. The last military use was in the Iran–Iraq war. SM injured over 100,000 Iranians and one-third are still suffering from its late effects. Its use was threatened in the early 1990s during the Persian Gulf War. Although, weapons and stockpiles of SM were discarded on land and into the ocean during and after the Second World War[6]. Periodically they come to surface during agricultural or fishing activities leading to serious injury. Extensive production and stockpiling during the Second World War along with the serious effects of SM, have led to the agreements to ban its production and use. These agreements include the 1993 chemical weapons convention (CWC) and agent destruction programs. Nonetheless, this chemical warfare agent is still regarded as threat for modern societies and remains a threat owing to its

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popularity in some countries that are not signatories to the CWC and to accidental exposures[7]. Besides their military use, SM and its analogues found their way into medical therapy. Their strong cytotoxic effects were found to be useful in the therapy of cancer[8]. The ease of availability of precursors, the simple method of synthesis and extremely stable nature of SM make it a weapon of choice by military and terrorist groups[9,10].

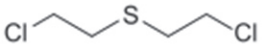
1.1 Physico-chemical Properties of SM

SM is a pale yellow, oily liquid having a molecular weight 159.08, specific gravity 1.27, viscosity 0.046 poise, refractive index 1.531, freezing point 14.4 °C that vaporizes at 25 °C and decomposes at 217.5 °C[11]. It is a liquid in cold and damp environment and easily vaporizes in warm dry environments. It is heavier than air with a density 5.6 times of air. It has an odor of mustard in the impure form but the pure form is colorless and odorless. It is sparingly soluble in water while soluble in fat and other common organic solvents. It easily penetrates ordinary clothes in vaporized form[12].

The half life for SM hydrolysis by water is about 3-5 minutes[13]. The rate of SM hydrolysis is not pH dependent and is not altered by ions such as Ag^{+}, Mn^{+}, Ca^{+}, and Fe^{+++}. Toxicity of SM is greater at higher temperatures, whilst at low temperature, mustard freezes, thus increasing its persistence. SM decomposes at higher temperatures to produce toxic compounds, including active lachrymators and hence disposal of material contaminated with SM should be undertaken with care.

1.2 Chemical Identity of SM

Chemical abstract name: Ethane, 1, 1`- thiobis [2-chloro] [after 1971], Sulphide, is [2 chloroethyl] before 1971. Other name: Bis [2-chloroethyl] sulphide; Chloro-2[2-chloro-thylthio] ethane; 2, 2-dichlorodiethylo sulphide; Yperite; Schwefel – Lost; 5–Mustard; Mustard gas; Levistein Mustard; Yellow cross Mustard;



2. MECHANISM OF ACTION OF SULFUR MUSTARD TOXICITY

2.1 DNA Damage

SM reacts with DNA by forming mono and bifunctional SM adducts[6]. N7 position of guanine is the preferred site for the production of these adducts, however, N1 position of adenine, N3 position of adenine and O6 position of guanine are also possible additionally[6]. O6- [2-ethylthioethyl] guanine is considered as a serious DNA lesion because human DNA repair machinery fails to eradicate the SM adduct at this position[6] and may cause significant mutagenic effects due to replication. Moreover, cell fate is vastly reliant on the quantity of SM alkylate DNA. DNA alkylation leads to variety of cellular responses consist of cell cycle arrest, terminal differentiation, apoptosis/oncosis or necrosis[14-16]. Genotoxic stress induced by SM stimulates DNA repair.

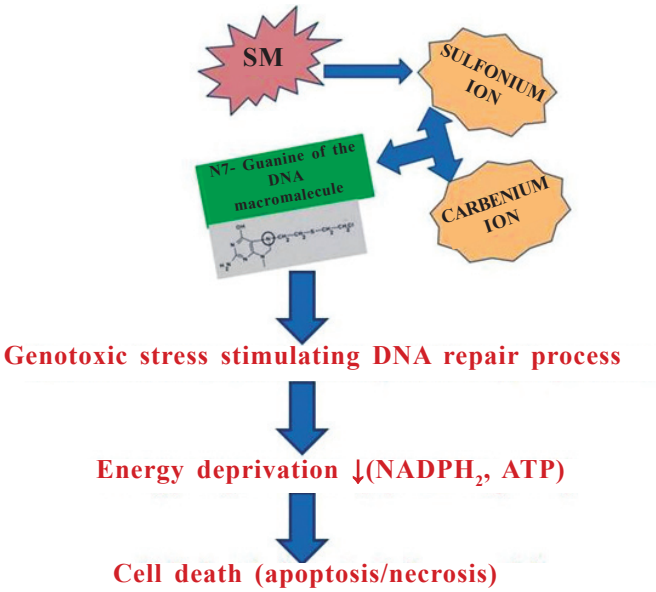


Figure 1. Diagrammatic representation of SM-induced genotoxic stress leading to cell death.

However, persistent DNA damage due to unsuccessful repair might result in programmed cell death either by terminal differentiation or via apoptosis[15].

2.2 PARP Signaling

Poly [ADP-ribosyl]ation of cellular proteins in association with marked exhaustion of nicotine adenine dinucleotide [NAD+] and adenosine triphosphate [ATP] has been noticed after SM contact and it depends on SM-induced activation of poly [ADP-ribose] polymerases [PARPs][17]. PARPs modulate SM induced cell death and thereby blister formation. PARP-1 and PARP-2 are activated by genotoxic stress in multicellular organisms. High SM absorption turns on PARP-1 with subsequent diminution of its substrate NAD+ and formation of [ADP-ribose] polymers[18]. PARP-1 is a caspase-3 substrate in the early phase of apoptosis[19] and lysosomal proteases in necrosis[20]. Low NAD+ inhibits glucose consumption and lactate formation[21,22]. NAD+ resynthesis further exhaust intracellular ATP stores[23,24]. Low ATP levels may lead to necrotic cell death[25,26]. PARP inhibition does not prevent cell death, although in the early hours after injury a limited degree of cytoprotection can be observed[27]. However, moderate SM exposure activates PARP-1 with low impact on NAD+ and ATP levels[27]. Thus, the conserved intracellular energy level favors beginning of apoptosis[6,28,29]. The serious role of intracellular ATP is authenticated by the result that PARP inhibition can shift necrosis to apoptosis[30].

2.3 Apoptosis

Apoptosis is defined by histo-architectural changes of the cell nucleus, where the chromatin material condenses to form compact figures from which smaller granules of chromatin [apoptotic bodies] are formed[31]. Other features are compression of cytoplasm, cutback in the membrane potential of mitochondria, intracellular

acidification, phosphatidyl serine exposure to cell surface, cell shrinking and membrane blebbing. The apoptotic machinery is motivated by the establishment of proteases known as caspases [cysteine-dependent, aspartate specific proteases][32]. Two major pathways have been described to trigger apoptosis, namely the extrinsic pathway [death receptor pathway] and the intrinsic pathway [mitochondrial pathway] within the cell. Interestingly, both pathways seem to be involved in SM-induced apoptosis[6].

Alkylation and cross linking of intracellular DNA with SM, leading to DNA damage is well documented[6,33]. SM-induced DNA damage results in PARP activation and depletion of cellular NAD+ and AT at the site of SM-induced skin injury, which results in necrotic cell death. Mild PARP activation does not disturb cellular energy levels and allows apoptotic cell death or recovery to occur [34]. Prelethal cell death reaction have been categorized into oncosis and apoptosis[35]. It has been reported that SM causes both oncotic (cellular swelling) and apoptotic [cellular shrinkage and nuclear condensation] type of pre-lethal cell injury, demonstrating a dose and time-dependent increase in DNA damage depicted by deoxyribonucleotidyl transferase [TDT]-mediated dUTP-digoxigenin nick-end labeling [TUNEL] assay[33,36] as shown in Fig. 2[16].

2.4 Calcium Signaling and Calmodulin

Intracellular Ca++ is mainly stored in the endoplasmatic reticulum [ER]. Ca++ release from this store is important in various cellular signaling corridors. SM can cause major ER stress with changes in Ca++ homeostasis and induction of cell death e.g. apoptosis[37]. Interestingly, SM induces a rise of intracellular levels of free Ca++ in adult and neonatal keratinocytes[38,39]. More recently, the key role of calmodulin 1 [CaM1] in SM induced apoptosis was demonstrated by Simbulan-Rosenthal[40] *et. al*. The exact mechanism of SM-induced rise in Ca++ is not clarified until today.

2.5 Nitric Oxide Signaling and Oxidative Stress

Fascinatingly, reactive nitrogen species [RNS] and peroxynitrite [ONOO–] have just been projected as key mediators of SM-induced toxicity[41,42]. Nitric oxide [NO] is produced by nitrogen oxide synthases [NOSs], which convert the amino acid l-arginine into NO and l citrullin. There are three types of NOSs namely [a] endothelial NOS [eNOS], [b] neuronal or brain derived NOS [nNOS], and [c] inducible NOS [iNOS]. eNOS can be triggered through translocation from the plasma membrane, where it is linked with caveolin. SM induced eNOS triggering from the caveolin scaffolding was first stated by Bloch[43], *et al*. Besides eNOS activation, Gao[44], *et al*. had demonstrated an up regulation of iNOS after SM injury. In combination, it appears possible that SM induced increase in free intracellular Ca++ and CaM-regulation could lead to NOS activation. SM induces concentration and time-dependent formation of iNOS[45] and activation of eNOS[16] due to translocation from plasma-membrane.

Oxidative stress has been proposed as a possible pathway for SM poisoning[46,47]. Glutathione is considered to play a crucial role in cellular and extracellular defense against alkylating agents, free radicals and oxidative stress[48] and is responsible for maintaining the thiol status of the cells. Reduction of the intra cellular GSH level decreases cellular resistance to oxidant insult[49,50]. Glutathione is an intracellular scavenger of SM[10]. Thus, SM may cause GSH depletion and enhances formation of reactive oxygen species [ROS][51,52]. It is not clear whether SM initiates peroxidation by direct interaction with lipids. However impairment of cell's natural protective system was proposed to be the cause for the peroxidation[53]. Cell death is thus proposed to be due to an accumulation of endogenous ROS (e.g. H_2O_2 accumulation resulting in hydroxyl and perferryl ion formation) leading to lipid peroxidation and irreversible membrane damage[54].

2.6 Inflammation

The histopathology of SM injured skin explained noticeable inflammatory reaction which point out the assembly or discharge of various vasoactive and chemoattractant mediators in the affected area[16,55]. Skin epidermocytes are the primary cells in contact with SM and are supposed to have a central role in the first phase of initiating toxic response. Several pathways have been

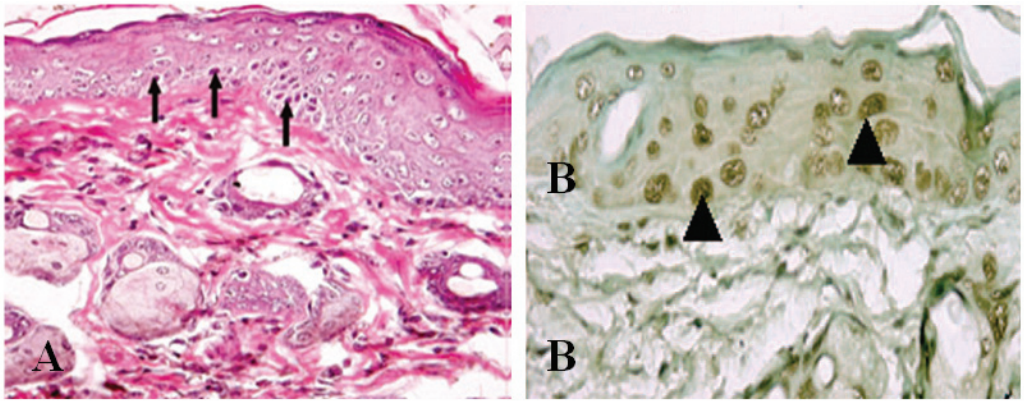


Figure 2. (A) Photomicrograph showing hyperplasia of epidermal cells, pyknotic nuclei of basal cells [arrow] and edema of dermis along with mild inflammatory reaction and (B) TUNEL positive cells in epidermis of mice skin section [arrowhead] 3 days after SM exposure.

recognized to be concerned in the gene expression of proinflammatory mediators. There is an immediate release of IL-1, IL-6, IL-8, TNFα and GM-CSF after SM exposure[16,56-58]. This cytokine pattern has strong chemotactic activity for neutrophils and macrophages. The NFκβ pathway and mitogen-activated protein kinases [MAPKs] are described to be substantially involved in the regulation of genes coding for inflammatory cytokines after SM injury[59,60]. More recently, the role of metalloproteinases, collagen degradation[61,62], platelet activating factor[63], and interaction of cytochrome P450 processes[64,65] are being investigated relative to the mechanism of action of SM.

2.7 Toxicokinetics

Mustards are very lipophilic therefore penetrates the epithelial tissues easily. The eye, respiratory tract and skin of unprotected persons will be most likely damaged after exposure to SM. 10–20% of total mustard that penetrates skin is fixed to the macromolecules and remaining 80–90% is rapidly transported away by circulation[66]. The distribution of SM is quick with a long terminal half life [$t1/2$ = 5.56 min; $t1/2$ = 3.59 h]. The volume of distribution at steady state [*V*dss] is 74.4 l. Whole body autographic studies with 35 S-labeled SM have shown that elevated radioactivity was detected in the nasal region, followed by the kidneys, liver, and intestines at all times studied after percutaneous or intravenous administration[67]. Two studies in rats revealed that conjugation with glutathione is more important than hydrolysis[68,69]. More recent investigations demonstrated that 60% of the dose is excreted in the 24 h urine. Thiodiglycol sulphoxide, 1,1-sulphonylbis [2- S [*N*-acetylcysteinyl] ethane], and 1,1-sulphonylbis [2-[methylsulphinyl] - ethane] or 1-methylsulphinyl-2- [2 [methylthio] ethylsulphonyl] ethane are the most prevalent metabolites[70,71].

2.8 Pathogenesis of Blisters

The primary cutaneous cell population targeted by SM is the basal cell of epidermis[10,16,55,72]. In animal model studies, the development of an apparent initial nuclear pathologic condition of basal cells of the stratum germinativum was followed by progressive cytoplasmic changes, leading to the eventual death of affected basal cells. Microblisters are observed to arise from focal areas of epidermal-dermal separation [Fig. 3 (a), (b)] in areas of widespread basal cell pyknos is [Fig. 2(a)], 24 to 48 hours after SM exposure, as seen by light microscopy[16]. This separation is dependent on the loss of integrity of basal cells and anchoring filaments[73]. Progressive changes reported in basal epithelial cells include formation of perinuclear or paranuclear vacuoles [Fig. 3 (a)], a decrease in nuclear staining intensity, cytoplasmic swelling, relocation of chromatin to the periphery of the nucleus, loss of chromatin, and pyknosis[16,74]. These changes are followed or accompanied by necrosis, vacuolization, or hydropic degeneration of the cytoplasm, while the extent of nuclear damage is dose and time related[16,54]. However, the pathogenesis of micro blisters is not fully understood.

Petrali[75], *et. al.* found indications that proteins of extracellular matrices of the basement membrane zone are affected during the development of SM induced skin pathology in hairless guinea pigs and postulated that they may contribute to the formation of micro blisters. Immunohistochemical staining for bullous pemphigoid antigen, a noncollagenous protein shared between basal cell hemidesmosomes and the lamina lucida, revealed a diminishing of bullous pemphigoid antigen reactivity at early times and subsequent loss of antigenicity at later time periods after an 8 minute SM vapor exposure (tissues were harvested at selected post exposure time periods up to 24 h). Laminin, the major glycoprotein of the lamina lucida, showed scanty immunolocalization at the later time periods, conforming to the structurally altered lamina lucida at micro blister lesion sites. The reactivity of Type IV collagen, a ubiquitous protein assigned to the lamina densa of basement membranes, was unaltered to specific antisera throughout prevesication and vesication time periods. The influence of these altered macromolecules on repair mechanisms following SM toxicity is not known.

IL-6 has been recognized as a way for inflammatory cell recruitment and its activation at the site of SM-induced skin injury[16,55,76]. A time and dose-dependent increase in the expression of IL-6 in early stages followed by a sharp

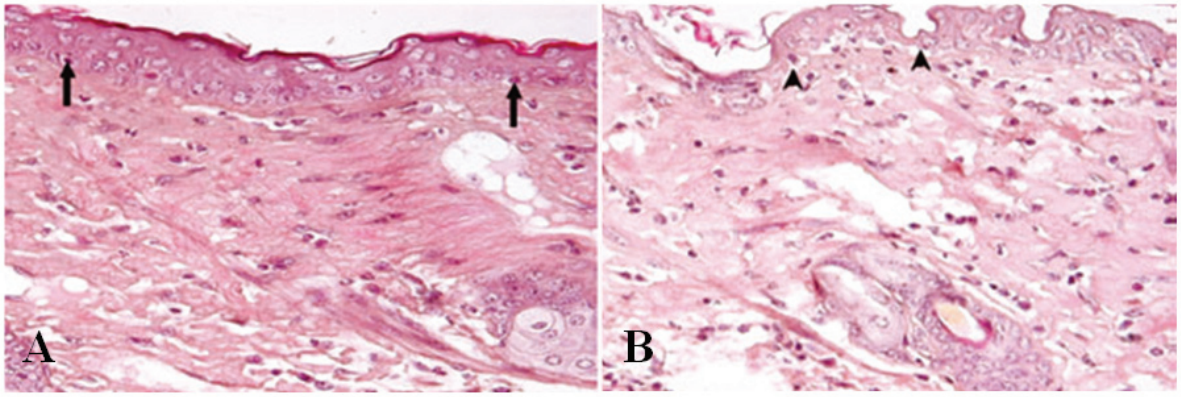


Figure 3. (A)1 h showing ballooning of epidermal cells, acanthosis in epidermis [arrow], oedema of dermis and a few transmigrated neutrophils and (B) 6 h showing ballooning of epidermal cells, dermoepidermal seperation [arrow head] and infiltration of inflammatory cells in dermis.

decrease in later stages in IL-6 expression in the mouse skin[16], mouse ear[77], weanling pigskin[58] and hairless mouse skin[78] after SM exposure that are consistent findings. Several in vitro and in vivo studies have suggested the role of inducible nitric oxide synthase [iNOS] induced NO synthesis, which is mainly produced by neutrophils and leukocytes[44,79,80]. A time-dependent expression of eNOS in endothelial cells of newly formed blood vessels and in granulation tissue during healing process of SM-induced skin injury, supporting the eNOS-induced NO synthesis has also been reported by us[16]. Further, angiogenesis involves complex sequential steps such as degradation of basement membrane by proteases, proliferation and migration/ invasion of endothelial cell, formation of capillary tubes and survival of newly formed blood vessels[16,81]. NO, is a prerequisite for the endothelial cell to enter into angiogenic cascade and increased NO production correlates positively with increased vascular density[16,82]. NO promotes angiogenesis, migration and proliferation of fibroblasts, epithelial cells, endothelial cells, and keratinocytes during the wound healing process[31]. This suggests that eNOS may play pivotal roles in all the events relating to wound healing, and the time dependent expressions of eNOS may be used possibly as a new marker for the age determination and in evaluating angiogenic activity for pharmaceutical screening of drug against SM-induced skin lesions.

Growth factors play a multitudinous role in wound repair process. Inflammatory stimuli triggers p38 MAP kinase which results in the release of TGF-α family ligands and activates the epidermal growth factor [EGF] receptor signaling, leading to enhanced keratinocyte proliferation, mitogenic to fibroblast proliferation and granulation tissue formation[31,83]. On the other hand, depletion of p38a MAP kinase activity suppresses EGF receptor signaling and downstream Erk MAP kinase signaling, as well as autocrine EGF-decreased proliferation. Our group has also reported a time-dependent correlation between proliferations of basal cells and TGF-α expressions by epidermal cells along with granulation tissue formation in SM-induced skin injury in mice. Fibroblasts and neovascularization strengthen the wound by increasing collagen and mucopolysaccharide production in the proliferative phase of the wound healing[16,84]. bFGF modulates fibroblast proliferation, its migration into damaged tissue, stimulates angiogenesis and affects cell differentiation[16,85]. We observed a time-dependent increase in bFGF expression, showing a significant correlation with fibroblast proliferation and tissue collagen synthesis in SM-exposed mice skin.

2.9 Models Systems for Screening SM Toxicity

Many *in vivo* studies have focused on euthymic hairless guinea pig[86-88] guinea pig, weanling pig (showing skin lesions similar to humans), rabbit, mouse ear vesicant model[89,90] or various mouse species (nude mice, euthymic hairless mouse etc.) as reviewed by Gerecke[91], *et. al*. as useful animal models for evaluating efficacy of various antidotes against SM-induced skin toxicity. But there are many variables that influence the differential severity of pathogenesis of SM induced lesions such as dose of SM, physical and chemical nature of diluents and duration of SM exposure[92]. Vijayaraghavan[93], *et. al.,* while screening various antidotes against SM toxicity, observed that SM was more toxic through percutaneous route compared to oral and subcutaneous routes. Our previous studies described the *in vivo* protection against systemic toxicity due to percutaneous exposure of SM diluted in PEG-300[52,53,93-97], DMSO[93] and acetone[98].

Animal models for evaluation of antidotes against SM-induced dermatotoxicity include weanling pig, hairless guinea pig, rabbit or hairless mouse[33,99-102]. However, hairless mouse models in which hr gene expression has been reduced or eliminated display phenotypes of hair loss and hyperproliferation of skin and formation of dermal cyst[103]. Additionally, lack of polyclonal and monoclonal antibodies and lack of probes and primers to DNA and RNA sequences in weanling pig and guinea pig limits the usefulness of these animals in the study of the pathophysiology of SM-induced skin lesions[104]. The standard mouse ear animal model for studying pathophysiology of SM-induced skin lesions have been shown to have altered reaction to injury with delayed inflammatory changes compared to mouse skin[78]. Animal models exposed with 2-chloroethyl ethyl sulphide [CEES; half mustard, used to produce SM like skin lesions] provides the initial steps for evaluation of therapies that need to be repeated using SM due to differences in the metabolism and the mechanism of action[105,106]. Thus, use of CEES as a stimulant of SM for antidote evaluation may not be appropriate. Recently, Lomash[10], *et al.* has designed and reported a mouse model for studying SM induced skin lesions. It has been suggested that Healing is modified by both systemic and local host factors[31]. Thus, damage to liver, kidney and spleen of test model on exposure to SM diluted in PEG-300 or DMSO will impair systemic host factors, which may Retard normal wound healing and will interfere in the wound-healing Process. On the other hand, there was no noticeable systemic injury observed in mice exposed to SM diluted in acetone, thus diminishing the systemic host factors that influence wound healing. Thus, the developed mouse model shall be resourceful, reliable, economical and suitable model for studying SM-induced skin lesions[10].

2.10 Target Organs of SM

Sulfur mustard is a blister agent (vesicant) that causes severe, delayed burns to (a) the eyes causing irritation, itching lacrimation, burning sensation, conjunctivitis and photophobia, marked hyperemia, perforation in the anterior chamber, corneal opacities, corneal ulceration, delayed recurrent keratitis, chronic conjunctivitis and keratoplasty[7,51,54,107], (b) the skin resulting in the formation of blisters which further turns to ulcers that heal very slowly and tend to become infected. If they do not heal, deep marginal pigmentation may develop afterwards[7,16,54,107] and (c) Inhaled SM injures the respiratory epithelium from the nasopharynx to the bronchioles leading to the symptoms like cough, chest pressure, sinus pain, and sore throat hoarseness have been observed on immediate

exposure[107,108]. Death after SM exposure generally occurs due to bronchopneumonia and secondary infections. Mice exposed to SM vapors showed sensory irritation during exposure and airway obstruction later[109]. SM damages cells within minutes of contact; however, the onset of symptoms is delayed until hours after exposure. Outsized contact to sulfur mustard may be lethal. SM is also damages the cells within the bone marrow thereby affecting the body's immune system. Finally, SM also affects a part of the nervous system and causes "cholinergic toxicity", evidenced by excessive saliva, tears and urine; gastrointestinal cramping and diarrhea; vomiting and meiosis. SM has been used as a chemical warfare agent to cause delayed casualties[107].

2.11 Current Treatment for SM Toxicity

Since, the medical management of SM exposure is not satisfactory, a considerable effort has been expanded in the development and evaluation of protective clothing and equipments[110]. Following exposure, rapid decontamination is essential. Ocular exposure will require rapid removal of the agent from the eyes by irrigating with water vapor. The use of laser debridement of burns has shown to increase the rate of burn resolution in animal models and may, therefore, be beneficial in SM induced skin injuries[111] Because no antidotes are available till date for SM toxicity medical management rely on prevention, decontamination and palliative treatment of signs and symptoms[112].

Drugs that were used for therapeutic or preventive measures against SM toxicity were anti-inflammatory drugs [e.g. Fluphenazine dihydrochloride, Indomethacin, Olvanil, Hydrocortisone etc.]; scavenger drugs [eg. Mercatopyridine-1-oxide, 6-Methyl-2- Mercatopyridine-1-oxide, 4- Methyl-2- Mercatopyridine-1-oxide, Hydrogen peroxide gel 3%, Dimercaprol]; Protease inhibitor [e.g. 1-[40-Aminophenyl]-3-[4-chlorophenyl] urea, N-[O-P]-L-Ala-benzyester hydrate]; PARP inhibitor [e.g. 3-[4-Bromophenyl]ureidobenzaide, Benzoylene urea] which were reviewed by Smith[113]. Hydrophilic formulation of CC2 [N, N'-dichloro-bis [2,4,6-trichlorophenyl] urea] was proved to be a favorable and preferred personal decontaminant by Vijayaraghavan[114], *et al.* Further, this formulation was fortified with aloe vera and betain for enhancing its wound healing efficacy without affecting its stability and decontamination activity[127]. Polyurethane sponges containing detoxification additives are currently being developed and evaluated for decontamination/ detoxification[116]. Amifostine, and its analogue DRDE-07 have shown significant protection against SM toxicity[95,97,117]. Some compounds showed good activity in vitro like L-nitroarginine methyl ester, diisopropyleglutathion ester etc[118-120]. but it's in vivo efficacy is questionable or very weak. The protective effect of flavonoids[121] and various antioxidants like trolox, quercetin and GSH[52] on the SM induced toxicity by percutaneous and inhalation routes have been investigated with promising results but require further evaluation. Wound healing is a biological process triggered by tissue injury and directed towards the restoration of tissue continuity and its function. The early phase of healing process is inflammation followed by fibroplasia and re-epithelialization and finally tissue remodeling[31]. These phases overlap and their separation is arbitrary. In SM injury to human skin, recognizable skin pathology does not usually occur for several hours to a day after exposure[91]. Oral pretreatment with drugs against SM has been studied in experimental models for accessing their protective efficacy[9,98].

It was a common belief that the stronger bactericidal effect of an antiseptic agent has more deleterious effect on living tissue, and earlier studies have shown impaired wound healing with the use of iodine[122,123]. Contradictorily, Mayer[124] showed that PVP-I ointment when used in conjunction with the newer gel-type occlusive dressings enhances the healing process. Povidone iodine has been reported to enhance angiogenesis and is a potent microbicidal[125]. A. vera is another important ingredient, which is widely used for its healing, soothing and moisturizing qualities. A. vera augments re-epithelialization and fibroplasia, thereby increasing the healing rate of mechanical wounds[126]. Topical application of Aloe vera gel may be beneficial for protecting the SM-induced skin lesions[98]. Lomash[55,] *et al*. has reported a formulation DRDE/WH-02 [consisting of PVP-I, A. vera gel and betaine] to be efficacious in showing increased intensity of re-epithelialization, fibroplasia and angiogenesis in SM-induced skin lesions. Moore[127] suggested that the delivery of iodine in the chronic wound induces influx of macrophages and T helper cells, which are considered to play a positive role in modulating wound healing. The increased influx of tissue leukocytosis by DRDE/WH-02 in mice model have resulted in early removal of tissue debris at faster rate, ultimately paving way for the initiation of reparative process.

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