Medical Countermeasures against Sulphur Mustard

Dr Uma Pathak

Defence Research and Development Establishment, Jhanshi Road, Gwalior-474 002

1. INTRODUCTION

Bis (2-chloroethyl) sulphide, commonly known as sulphur mustard (SM), is a highly toxic chemical warfare agent. SM is an alkylating agent with cytotoxic, mutagenic and vesicating properties. Its use on the battlefield results in debilitating injuries to skin, eyes and the respiratory system. Due to its multiple toxicological properties, it is also known as “king of chemical warfare”. Germans first used this chemical as a warfare agent in 1917. Many nations in the world have the ability to produce sulphur mustard and possess large stockpiles of this agent. By virtue of its deadly toxic action and effectiveness, it is an agent of choice in chemical warfare. Although the threat that chemical weapons will be used for military purposes is regarded to decrease in recent years, the risk of terrorists its use by is considered to have increased. This chemical is regulated under Chemical Weapon Convention (CWC) and is included in Schedule 1 category.

While many of the toxic manifestations that follow HD exposure to cells and tissues have been defined, the underlying mechanisms of pathology remain elusive. Various biochemical studies led to significant inroads for our understanding of the toxic mechanisms, but the therapeutic approaches were futile. Nine decades of research produced numerous hypotheses on the pathomechanism of sulphur mustard. Early investigations led to the acid liberation theory, suggesting that sulphur mustard is hydrolysed within cells to form hydrochloric acid. The theory was soon rejected because vesicant action does not run parallel to the rate of acid liberation. Reactions of sulphur mustard with proteins and inhibition of several enzymes, especially hexokinase, were later assumed to be the most important biochemical lesion. However, the level of alkylation needed for enzyme inhibition in vitro does not correlate with the vesicant doses in vivo. Additionally, inhibition of hexokinase due to alkylation should be completed within minutes as would be expected for the resulting cellular damage. In contrast, tissue damage in vitro and in vivo caused by sulphur mustard appears with a substantial delay. Other theories include the depletion of glutathione and lipid peroxidation. All the above mentioned theories are not in agreement with the typical delay of the damage after exposure to sulphur mustard. Nevertheless, some of the biochemical changes which led to these theories are recognized as components of the proposed current theory of sulphur mustard cytotoxicity.

Figure 1. Reaction of SM with DNA (N 7 Guanine).
The present most accepted theory of the basis of toxic effects by the mustard family proposes that these are consequences of alkylation reactions with cell constituents, mainly with DNA, but also RNA, proteins, and lipid membranes. These reactions may result in physiological, metabolic and genetic failure of cellular functions. Each 2-chloroethyl side chain of the sulphur mustard molecule undergoes first order (SN1) intramolecular cyclisation with the release of the chloride ion. The ethylene sulphonium cation intermediate opens to form the highly reactive carbonium ion which reacts immediately with nucleophiles, such as DNA, RNA, proteins and other molecules.

2. REACTION OF SM WITH DNA (N7 GUANINE)

In principle, there are three possible strategies for prevention of injuries caused by SM. The first is to attempt to prevent SM from alkylating critical target molecules; the second, to attempt to reverse alkylation reactions after they have occurred; and the third, to attempt to prevent or reverse the secondary biochemical consequences of alkylation that occur during the latent phase of injury, before the onset of irreversible cell and tissue destruction. Serious technical and/or practical barriers are associated with each of these approaches.

Prevention of alkylation reactions is technically feasible and relatively straightforward; molecular design approaches can be predicated on a fairly well-established knowledge of SM chemistry. Indeed, prevention of alkylation can readily be demonstrated. However, in practice, this strategy has proven quite difficult because of the speed with which SM reacts.

The second strategy, reversal of alkylation reactions, is considered technically unfeasible because the stability of most of the SM adducts that are formed is such that, under physiological conditions, alkylation reactions are probably irreversible.

The final strategy, reversal of secondary consequences of alkylation, is considered feasible but technically demanding, in that it may require simultaneous intervention in several deleterious processes that contribute to the injury process. Moreover, interventions at this level must be developed on the basis of a detailed understanding of the biochemical pathways leading to cell death and tissue destruction--and this understanding is, at present, incomplete. This aspect may require future attention.

Based on the above principles and proposed concepts of SM toxicity, several distinct mechanistic classes of drugs can be envisioned that may by themselves prove effective against SM toxicity or may constitute one element of a multi drug therapy. It is important to emphasize that many of these classes represent theoretical possibilities.

The first class: SM scavengers, comprises those compounds that prevent alkylation of critical targets by reacting either with the cyclic ethylene sulfonium intermediate of SM, or with the sulfide form of SM, thereby reducing the amount of SM available to react with critical targets. As will be discussed further below, the majority of compounds that have been examined react with the ethylene sulfonium intermediate of SM and, thus, fall into this class. Representative members of this class that have been tested include sodium thiosulfate, cysteine, and numerous other sulfur compounds.

Second class of compounds, DNA repair agents, consists of compounds that induce or otherwise promote endogenous enzymatic DNA repair mechanisms. Compounds of this type can be viewed as indirectly reversing the alkylation reactions of SM with DNA. Although the alkylation reaction is not actually reversed, its consequences effectively are reversed, since the net effect of such treatment is that alkylated bases are replaced with normal ones. Although such compounds are possible in theory, no candidates of this type are known.

The rationale for the third class of compounds, nicotinamide adenine dinucleotide (NAD+)-level stabilizers, is based on the phenomenon of SM-induced NAD+ depletion, which is proposed to play a central role in energy depletion and acute cell death. Some representatives of this class have been tested, including inhibitors of the enzyme poly (ADP-ribose) polymerase (PADPRP) and some metabolic precursors of NAD+, such as nicotinic acid. Utilising a similar rationale, compounds in the fourth class, energy-level stabilizers, allow cells to maintain normal ATP levels despite NAD+ depletion; for example, by providing alternative carbon sources (i.e., other than glucose) that are not processed via the glycolytic pathway.

Depletion of cell energy supplies is presumed to lead to elevation of intracellular calcium, a process that may also be brought on by depletion of intracellular glutathione (GSH) levels. Elevated calcium, in turn, is thought to result in the activation of autolytic enzymes, including proteinases, phospholipases, and nucleases. Thus, compounds in the fifth class: calcium-level stabilizers, interfere with calcium uptake or transport mechanisms, or otherwise antagonize increases in intracellular calcium levels. Likewise, compounds in the sixth class, autolytic enzyme inhibitors, are envisioned to limit cell damage by interfering with the activities of these enzymes. Such compounds may also be effective in limiting extracellular tissue damage caused by the release of proteinases. At present, there has been no systematic examination of the efficacy of compounds in these two classes.

Antioxidants are a seventh mechanistic class that may be of importance. It has been proposed that reactive oxygen radicals may arise as a result of GSH depletion and may contribute to activation of autolytic enzymes through elevations of intracellular calcium and/or to membrane damage as a result of increased lipid peroxidation. At present, the involvement of such a mechanism in SM injury remains speculative, and, with the exception of investigations of certain sulfur compounds whose mechanisms of action may be more closely associated with their mustard-scavenging activity than with their antioxidant activity, there has been...
little study of the effect of antioxidants on the SM injury.

Compounds in the eighth class, cell-cycle regulators, are hypothesized to act by slowing the cell cycle in order to prevent lethal consequences of aberrant cell-cycle progression. Such compounds would be expected to be relatively ineffective by themselves, but may be effective when used in concert with other drugs that prevent acute cell injury without directly affecting DNA damage. No members of this class have been tested against SM in mammalian systems.

Compounds in the final class, anti-inflammatory drugs, may limit tissue damage by interfering in secondary inflammation caused by cell damage. Again, such drugs may not be completely effective by themselves, but may enhance the protection achievable with other drug classes. A limited number of tests of anti-inflammatory drugs have been performed.

Acute poisoning with chemical weapons may induce severe toxicity and death, requiring immediate therapy. But, no really effective antidote against sulfur mustard is available so far. Since these types of poisonings are generally rare, antidote against SM is an “orphan drug”. Due to the lack of economic benefits, there has been limited research interest by pharmaceutical companies to develop antidotes against such agents. Medical interventions are limited to symptomatic treatment designed to contain and prevent infection of lesions and to promote healing. Hence, development of effective medical pretreatments and treatments for SM poisoning are urgently required.

Though, several antidotes have been evaluated for reducing the systemic toxicity of SM. But, these have given only limited protection. Many investigators have proposed sodium thiosulfate, which significantly decreased lethality, prolonged survival time, antagonized decrease in body weight and lessened the degree of pathological organ damage induced by SM. Despite these positive results, sodium thiosulfate has several disadvantages that have precluded its practical use as an antidote for systemic toxicity of SM in a field situation.

One of the main charters of duties of DRDE, Gwalior is to develop medical defence against toxic agents. For this reason, and recognizing the increasing threat of terrorist use of chemical warfare agents, the responsibility for research into medical countermeasures against this weapon is of primary interest to DRDE.

During The drug development programme against toxicants undertaken by DRDE, S-((u -Aminoalkylamino) alkyl aryl sulphide dihydrochlorides \[H_2N(CH_2)_nNH(CH_2)_2SR\] Particularly S-2-(2-aminoalkylamino)ethyl phenyl sulfide dihydrochloride has shown promising efficacy against Sulfur Mustard (SM). This compound provides excellent protection when given prophylactically through oral route. It’s LD$_{50}$ (oral) is 1247 mg/kg and P. I. value is 22.8 (mouse model). The effectiveness of DRDE-07 against Sulfur mustard has also been supported by a no. of in vitro and in vivo studies for various pharmacological and biochemical parameters. Not only this but, some of its S-alkyl substituted analogues such as DRDE-30 \[H_2N(CH_2)_nNH(CH_2)_2SC_3H_7\] and DRDE-35 \[H_2N(CH_2)_nNH(CH_2)_2SC_4H_9\] have also been found very effective against SM.

At present these compounds are being investigated for their efficacy against Sulfur mustard and nitrogen mustards for different pharmacological and biochemical parameters.

REFERENCES