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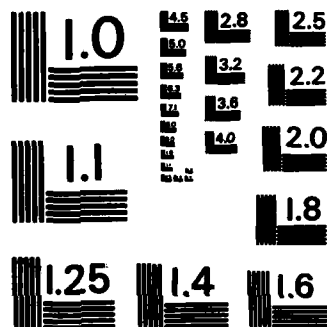
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THIOSULFATE AS AN ANTIDOTE TO MUSTARD POISONING
A Review of the Literature

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DIVISION OF RESEARCH SUPPORT

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**Thiosulfate as an Antidote to Mustard Poisoning: A Review of the Literature
—McKinley, McKinley, and McGown**

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21. ABSTRACT (Continued)

→ Sodium thiosulfate appears to be non-toxic and is predicted to remain in the extracellular fluid where it is quickly excreted. Sodium thiosulfate reacts with cyclized mustards and is most effective against mustards that cyclize rapidly (SN_2 reactors) in the extracellular fluid. Post treatment with sodium thiosulfate is ineffective due to the rapid reaction of mustards in the body. Simultaneous injection with 200 mg or more of sodium thiosulfate per mg of mustard provided some protection. Comparable dosages of sodium thiosulfate injected 10 to 45 min. before mustard exposure was an effective antidote to SN_2 reacting mustards. Effectiveness of topical application of sodium thiosulfate apparently is not known. ←

ABSTRACT

This report reviews the multidisciplinary literature (from World War I to the present) on sodium thiosulfate as an antidote to sulfur and nitrogen mustards. Intramolecular cyclization of sulfur and nitrogen mustards yields the active form which is responsible for the varied effects upon an organism. Symptoms associated with mustard poisoning can be local (eyes, skin, and respiratory tract), systemic, or both. The toxic effects include cell death, inhibition of mitosis and decreased tissue respiration. A great deal of evidence indicates that the toxic effects are directly related to the alkylation of DNA. Sodium thiosulfate appears to be non-toxic and is predicted to remain in the extracellular fluid where it is quickly excreted. Sodium thiosulfate reacts with cyclized mustards and is most effective against mustards that cyclize rapidly (SN₂ reactors) in the extracellular fluid. Post treatment with sodium thiosulfate is ineffective due to the rapid reaction of mustards in the body. Simultaneous injection with 200 mg or more of sodium thiosulfate per mg of mustard provided some protection. Comparable dosages of sodium thiosulfate injected 10 to 45 minutes before mustard exposure was an effective antidote to SN₂ reacting mustards. Effectiveness of topical application of sodium thiosulfate apparently is not known.

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PREFACE

The Chemical Defense Office at U.S. Army Medical Research and Development Command expressed interest in sodium thiosulfate as an antidote for mustard gas. We encountered many difficulties in obtaining information. Documents were widely scattered and poorly referenced; the subject was classified until the mid 1940s. We are unaware if any classified documents exist at this time; we obtained those which had been declassified. Initially, we did a series of computerized searches: Biosis (1969 to 1981), NTIS (1964 to 1981), SSIE (1978 to 1981), Scisearch (1978 to 1980), Medline (1966 to 1981); these data banks have indexed the literature for the years given in parentheses. We also requested information from the Defense Technical Information Center (personal communication, September 1981). The results of these searches had limited benefit because the data banks did not cover studies done before 1964. We, therefore, obtained a large portion of the material by manual search. Only publications in the English language were reviewed. Our searches failed to produce any articles linking sodium thiosulfate and mustards in other languages. We are not aware of any comprehensive review of sodium thiosulfate as an antidote to mustard in any language, and hope others will benefit from our efforts. A review of the subject is contained in these pages, documented by the References and supplementary Bibliography.

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THIOSULFATE AS AN ANTIDOTE TO MUSTARD POISONING

A Review of the Literature

—McKinley, McKinley, and McGown

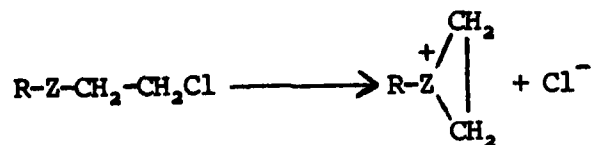
Chemical agents may be stockpiled around the world. The potential dangers have prompted renewed interest in antidotes for these agents. One agent about which the U.S. Army is concerned is mustard gas. Research has been done on mustards and mustard antidotes since mustard gas was introduced into the battlefield by the Germans during World War I (1). Interest in mustards and their antidotes has remained high, in part, because mustards were found effective in cancer chemotherapy treatments. Classified research has been done under government contracts. Non-classified research has been conducted in multidisciplines. This has caused the literature to be spread among many journals and government reports—some of which date from the World War I period and are not easily accessible.

The purpose of this report is to review the literature on sodium thiosulfate with respect to its efficacy against mustard poisoning. This task was undertaken after the Chemical Defense Office of U.S. Army Medical Research and Development Command specifically expressed interest in thiosulfate. The first portion of this paper will acquaint the reader with the mechanisms of mustard actions. The second portion is devoted to thiosulfate, particularly its efficacy in treating animals and humans exposed to mustard poisoning.

MUSTARDS

Common reaction

Mustards are alkylating agents and extremely toxic (2). The two classes of interest, sulfur mustards and nitrogen mustards, have structural similarities and a basic chemical reaction in common. The key reaction is the intramolecular cyclization in a polar solvent (such as water) to form a cyclic onium cation and a free chloride anion (3-5). An example of this cyclization is shown below.



Z = Nitrogen or Sulfur

The cyclized form is responsible for the varied effects of the sulfur and nitrogen mustards (3). Because the mechanism of action of nitrogen and sulfur mustards is similar, they will be considered together and termed mustards.

Skin Penetration

In their pure state, mustards are oily lipophilic liquids with little solubility. Thus mustards easily penetrate skin and mucosal surfaces (6). When the skin is contaminated with mustard, 3 to 5 min elapse before penetration of the epidermis occurs (6). During this time period and up to 15 min after exposure, most of the mustard can be removed from the skin with an organic solvent. This procedure must be done properly to prevent spreading the contamination (7). Evaporation of the mustard from the skin's surface occurs up to 60 min after exposure. More mustard evaporates than penetrates into the lower tissues (8), provided nothing inhibits the evaporation process (9). Nagy et al (10), exposed human skin to the concentrated vapors of three mustards -- bis (beta-chloroethyl) sulfide (H), ethyl-bis (beta-chloroethyl) amine (EBA), and tris (beta-chloroethyl) amine (TBA). They found that skin penetration was in the order of $\mu\text{g}/\text{cm}^2$ and that it was proportional to the temperature. Furthermore, penetration rates of H, EBA, and TBA were more dependent on the volatilities of the mustards than on their chemical structures. It was also found that the penetration rate of mustards into human skin varied little among individuals; however, there was a large variation in the sensitivity of individuals. The sensitivity of people exposed to concentrated vapors of dichloroethyl sulfide varied by as much as 600 times with respect to erythema formation (11).

Local and Systemic Effects

The symptoms associated with mustard poisoning can be both local and systemic (12). Literature on the actions and pathology of mustards is voluminous and only a brief summary is presented in this paper. Additional references not cited in the text have been listed in the Bibliography.

The three areas where local symptoms can be observed readily are the eyes, skin, and respiratory tract (12). Symptoms and severity vary according to the type and amount of mustard exposure. Frequently the eyes develop conjunctivitis, which may become so severe that they are swollen shut. Other ocular effects include lacrimation and necrosis of the cornea (12-15). In the respiratory tract mustards cause edema and necrosis of the trachea and bronchial epithelium, in addition to congestion of the lungs. Dermal lesions may include erythema, edema, vesication, and necrosis (12,14,15). Reactions are more severe in moist skin than in dry skin (10). This may be a result of reduced

mustard evaporation and accelerated cyclization of the mustard in the localized area.

For the mustard to affect an individual systemically, it must first make its way into the blood stream (12). As soon as it invades the blood, it is quickly distributed throughout the body. The mustard rapidly disappears from the blood (16) by entering the tissues, reacting with the blood or, to a small degree, being excreted in the urine (12). Proliferative tissues are the most sensitive to mustards (3) and include the reticuloendothelial system and bone marrow (14,17,18). The toxic effects include the death of cells, inhibition of mitosis (3), decreased tissue respiration (19), and other metabolic disorders (3).

Biological Reactions

Cyclized mustards react to varying degrees with almost every biologically important functional group (20). The list of functional groups includes alpha-amino, imidazole, sulfhydryl, sulfide, phenolic, epsilon-amino, imino groups of amino acids and peptides, inorganic phosphate, glycerophosphate, hexose phosphates, and the carboxyl and amino groups of proteins (3,21-23).

According to Gilman et al (2), a great deal of evidence indicates that the cytotoxic and other effects of mustards are directly related to the alkylation of DNA. Of particular susceptibility to alkylation is the 7 nitrogen in guanine. Both monofunctional mustards (those having one reactive group) and bifunctional mustards (those with more than one reactive group) form covalent bonds at this point. Other sites in the purine and pyrimidine bases of DNA are susceptible to alkylation, but to a lesser degree. The bifunctional mustards are largely cytotoxic in action, whereas the monofunctional have a larger capacity for mutagenesis and carcinogenesis. This indicates that the permanent changes in the DNA structure by monofunctional mustards pose a lesser threat to cell survival than the cross-links of DNA strands caused by bifunctional mustards. Of primary importance for a cell's resistance to alkylation may be its DNA repair system. The alkylation of a single strand of DNA could be repaired with relative ease; however, cross-links between the two strands of DNA would be much harder to restore. The increase in cross-linking of DNA strands would cause a breakdown of the DNA, especially in higher dosages. In addition to the DNA repair system, factors that may effect a cell's resistance to alkylation are the loss of permeability in the cell's membrane and the occurrence of more nucleophilic substances in the cell that can compete with DNA for alkylation (2).

Enzyme Inhibition

Many enzyme systems have been investigated for potential inhibition

by mustards (3). Most were not affected but some of the enzymes were found sensitive to mustards in vitro. Proteases and phosphokinases appear to be particularly sensitive (24). A significant number of the enzymes require mustard doses several times the lethal concentration for full inhibition to occur (24,25). Mustards cause inhibition either by reacting with the enzyme's active site or by combining with different reactive groups on the protein (19,25). The mustard effect is essentially irreversible (25). Barron et al (25) found an enzyme's sensitivity is dependent upon the tissue in which it is located. Choline oxidase, for example, was inhibited in rat kidney but not in rat liver.

THIOSULFATE

Toxicity

As a drug, sodium thiosulfate appears to be relatively non-toxic. Schultz et al (26) considered sodium thiosulfate to be essentially innocuous at intravenous doses up to 1,200 mg/kg in the dog. Bonadonna and Karnofsky (27) found no toxic effect in humans given a single dose of up to 400 mg/kg over a 10 to 15 min-period. In 1943, Litwins et al (28) injected two volunteers intravenously with 3100 and 3800 grains sodium thiosulfate, respectively, over a two-week period and monitored them for 4 months. No morphological or chemical changes were detected in the blood. However, in subsequent studies they found that large doses of sodium thiosulfate increased bleeding time. Eleven people injected with 50 cc of 50% sodium thiosulfate solution demonstrated a significant increase in bleeding time from an average of 10 min to an average of 23 min over a 3-day period. No explanation was obtained for the increased bleeding time.

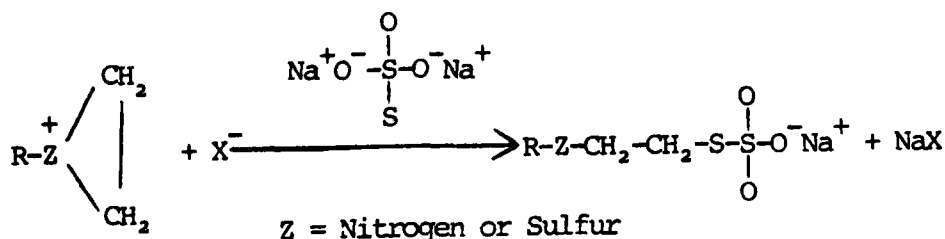
Distribution

Sodium thiosulfate injected intravenously is rapidly distributed throughout the extracellular fluid (29). The half-life of thiosulfate in the blood stream of dogs is approximately 25 min. Investigators (29-31) predict little thiosulfate will enter cells due to its negative charge and the lack of a specific transport system in the cell membrane. In the kidneys, there is no detectable tubular reabsorption and glomerular filtration clears the body of thiosulfate in a relatively short time (29,31). This is a disadvantage when high concentrations are desired for prolonged periods of time. To bypass this problem, methods to slow the rate of thiosulfate excretion have been studied. Increased levels of insulin and glucose have been found to retard the excretion (28).

Efficacy of Thiosulfate Treatment against Mustards

General reaction. Early in the search for mustard antidotes,

chemists recognized that thiosulfate reacted with the cyclized form of mustard in vitro (32,33). An example of this reaction is given below.



Some mustards cyclize slowly in a polar solvent, thereby limiting the alkylation rate. These mustards are classified as SN_1 reactors. Other mustards (e.g., HN_2) cyclize rapidly and are classified as SN_2 reactors. Their alkylation rate depends upon the concentrations of both the mustard and the target molecule. Because SN_1 type mustards are relatively more stable than SN_2 mustards, more of the former penetrate cells and react with intracellular targets (34,35). Thiosulfate reacts only with cyclized mustard and because it does not appear to enter cells, it is most effective against mustards that are SN_2 reactors (28).

Post Treatment With Thiosulfate. A number of studies have been performed to evaluate thiosulfate's usefulness against the systemic effect of mustards. Gilman (32) noted that mustard injected in its cyclic form reacted within the body so rapidly that subsequent injection of thiosulfate had no protective effect. Hatiboglu (36) found no significant protection when sodium thiosulfate was injected intraperitoneally 5 min after intraperitoneal injection of bis-(2-chloroethyl) methylamine (HN_2). After mustard enters the circulation, it either penetrates the cells or cyclizes and reacts so rapidly that subsequent treatment with thiosulfate is ineffective (37). As soon as mustard reacts with proteins or nucleic acid, thiosulfate does not influence the development of toxic effects (35).

Simultaneous Treatment With Thiosulfate. Several factors influence the efficacy of thiosulfate against mustards. These include the time interval between thiosulfate and mustard introduction (30), the concentration of thiosulfate relative to mustard (27) and the route of administration of both (30). The rapid clearance of thiosulfate from the body and the need to have it present in the extracellular fluid (38) before mustard exposure limit the allowable time interval between thiosulfate and mustard administration. Several studies have been conducted in which thiosulfate and mustard were injected simultaneously. In a study using dogs, thiosulfate and HN_2 were injected at the same site and protection was observed when 200 times as

much thiosulfate as mustard was used (26). The simultaneous injection at the same site, intravenously and intra-arterially, allowed rapid mixing of the two with subsequent neutralization of the mustard. Simultaneous intravenous injections of thiosulfate and mustard at separate locations have also been tried. Dogs, were protected from the systemic toxic effects of HN_2 provided the thiosulfate dose was 200 times that of the mustard (39). However, in a similar study using dogs, thiosulfate failed to protect the hematopoietic system (38). A possible explanation for the failure in that study was that the mixing of thiosulfate and mustard was inadequate to allow sufficient neutralization (38). In an attempt to isolate limbs or organs for mustard treatment, a system was set up where mustard was injected into the artery supplying the limb or organ with simultaneous injection of thiosulfate into the vein exiting the limb or organ. In two separate studies of this type, systemic protection was given when 200 mg or more of thiosulfate per mg of HN_2 was administered (40,41). In a study on mice where 500 mg/kg thiosulfate was injected intravenously followed by immediate injection of 25 mg/kg HN_2 , intraperitoneally or subcutaneously, survival time was significantly increased over the controls (36). The results from these studies indicated that thiosulfate is a systemic antidote to mustards when the two are injected simultaneously.

Pretreatment With Thiosulfate. Pretreatment with sodium thiosulfate allows neutralization of cyclized mustard in the extracellular fluid and at the cell surfaces. The mustard that does not cyclize rapidly (i.e., SN_1 mustards) in the extracellular fluid enters the cell where it subsequently cyclizes and reacts (5). Since thiosulfate does not enter the cells to any appreciable extent, it is ineffective in preventing mustard reactions with intracellular target molecules (5). However, if enough of the mustard is neutralized outside the cell, systemic toxic effects can be reduced or prevented (34).

Gilman's group (32,33) found that animals were protected when pretreated with a high concentration of thiosulfate prior to nitrogen mustard. When 1500 to 3000 mg/kg of sodium thiosulfate was injected intraperitoneally 15 to 60 min before intraperitoneal injection of a lethal dose of HN_2 in mice, 70 to 100% of the treated animals survived, the best protection being obtained when mustard was injected 15 min after the thiosulfate (38,42). In dogs, 3000 mg/kg thiosulfate was injected intraperitoneally before intravenous and subcutaneous injection of HN_2 . The best protection was observed when thiosulfate was administered 15 to 45 min before intravenous injection or 30 min before subcutaneous injection of mustard (42). When 200 mg/kg of thiosulfate was injected intravenously 30 min before intra-arterial injection of mustard (2 mg/kg), 6/6 dogs survived compared to only one control (43). In both of the foregoing cases with dogs, thiosulfate afforded good systemic protection. There were, however, local effects

around the mustard injection site (42,43). This was probably caused by the high local mustard concentration relative to thiosulfate immediately after injection (40) and increased cell penetration before cyclization. Thiosulfate also has been investigated for topical protection. Hibioglu (36) reported that when 10% sodium thiosulfate solution was applied to skin wounds of rabbits exposed to 0.015 to 0.1% HN_2 solutions, local effects were significantly reduced.

Some of the studies have yielded less favorable results. In one study where dogs were infused with 400 mg/kg thiosulfate for 30 min before 2.0 mg/kg HN_2 were injected at the same site, all the animals died (26). In another study, rats and rabbits were pretreated with thiosulfate intravenously before HN_2 and 1-methyl-1-(beta-chloroethyl) ethylenimonium picryl sulfonate (CPS) were administered intravenously. There was no significant protection against the toxic effects of HN_2 , but there was good protection from CPS effects (27). Gilman et al (33) used thiosulfate to protect rabbits against a mustard gas. When a lethal dose of the mustard was applied cutaneously or subcutaneously after intravenous pretreatment with thiosulfate, some systemic protection was seen. There was much less protection in pretreated rabbits when both the mustard and thiosulfate were injected intravenously (33).

Studies on Humans. Similar studies have been performed on humans. Pretreatment with 200 mg/kg thiosulfate intravenously gave protection from both intravenously administered HN_2 and CPS, with greater protection against CPS (27). Several subjects were infused with 2.8 to 8.0 mg HN_2 intra-arterially daily over a 2 to 6-hr period with simultaneous infusion of 250 mg thiosulfate per mg HN_2 . The hemopoietic system was protected, even though the levels of HN_2 used would have normally been deleterious (40). Systemic protection from topical application of mustards was provided by subcutaneous injection of thiosulfate up to 15 min after mustard exposure, although the local effects were unaltered (33).

Thiosulfate in Combination

Other antidotes. Some attempts have been made to improve the systemic protection of thiosulfate by using other possible antidotes in combination with the thiosulfate. In one study, sodium thiosulfate, cysteine, and methenamine (1000 mg/kg of each) were injected intraperitoneally, separately, and in combination into mice 30 to 40 min before a lethal dose of nitrogen mustard (10 mg/kg) (44). Cysteine plus thiosulfate with or without methenamine reduced the mortality rate to 10 to 11% with the controls having 100% mortality. The administration of individual drugs or other combinations was associated with 80 to 100% mortality from concomitant nitrogen mustard. Connors et al (34) injected rats intraperitoneally with 2 g/kg thiosulfate and 1 g/kg cysteine separately and in combination before the

intraperitoneal injection of an LD₅₀ dose of HN₂ and merophan (o-di-2-chloroethylamino-DL-phenylalanine) mustards. Cysteine was effective against both mustards. Thiosulfate was effective against HN₂ (an SN₂ reactor), but not against merophan (an SN₁ reactor). Cysteine was considered effective against both mustards by entering the cells and increasing the SH level. Thiosulfate and cysteine used in combination gave a slight increase in protection against HN₂ over what either one did separately. However, they provided little protection against merophan, much less than cysteine did alone. It was suggested that thiosulfate interfered with cysteine entering the cells, so cysteine like thiosulfate remained outside the cells and would only be effective against mustards that are SN₂ reactors. Callaway and Pearce (45) studied the efficacy of sodium thiosulfate alone and in combination with sodium citrate (thiocit) against bis (2-chloroethyl) sulphide (HD) in rats. They injected mustard gas subcutaneously into the loose skin of the right groin, with various routes of thiosulfate introduction. Injection of 300 mg/kg thiosulfate intraperitoneally 10 min before 6.27 mg/kg HD gave good protection with only 2 of 10 rats dying compared to all the controls dying. When the same dose of thiosulfate was infused subcutaneously over 33 min immediately following injection of HD, it gave very little protection. Oral administration of thiosulfate before HD exposure was also attempted, with poor results. When 2750 mg/kg thiocit was injected intraperitoneally 10 min before or after 6.75 mg/kg mustard gas, all rats survived. The intravenous thiocit proved to be more effective than intravenous thiosulfate as an antidote to HD. However, oral thiocit was no better than thiosulfate.

CONCLUSIONS AND RECOMMENDATIONS

Generally, it can be concluded from these studies that thiosulfate is an effective antidote to the systemic effects of SN₂ mustards if sufficient thiosulfate is injected intravenously or intraperitoneally 10 to 45 min before intraperitoneal, subcutaneous, and topical mustard exposure. Thus, it would be a practical systemic antidote, only in the event of advance warning of an attack. The thiosulfate within the extracellular fluid would not be expected to be useful in preventing skin lesions produced by mustards. Topical application of thiosulfate before or shortly after mustard exposure may prevent or reduce the local effects, although research needs to be done in this area before any conclusions can be drawn.

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