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REVIEW ARTICLE

THERAPEUTIC STRATEGIES AGAINST SULPHUR MUSTARD INDUCED TOXICITY: AN UPDATE

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ABSTRACT

Despite 90 years of research, there is still no antidote for sulphur mustard (SM). As the mode of its action is still lacking, no specific treatment is so far known against SM induced systemic toxicity. This fact is especially crucial when we consider that probably at least a dozen countries have SM in their arsenals today. Development of an effective prophylactic or therapeutic antidote is an immediate requirement for the destruction of SM. This review will focus on the therapeutic strategies investigated, and novel therapeutic targets currently under investigation for transition to advanced development.

Key Words: *Antidote, Toxicity, Sulphur mustard.*

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1. INTRODUCTION

During the World War I (WWI) the nations of the world use chemical as weapon ; killing people with flying metal was one thing but launching cloud of deadly chemical was another; effects of which were neither predicted nor controlled. Sulphur mustard (SM) was first used at the time of WWI as a chemical weapon, it is highly poisonous it could easily penetrate protective mask that were used during that time. SM causes slow and painful death and often causes cancer.

In 1925, the Geneva Convention has banned the use of chemical weapon but the production of SM continued in many countries. However, in recent years the use of chemicals as weapon has decreased for military purpose but use by terrorist on army and civilians has increased (1). Chemical weapons produce acute poisoning which may lead to severe toxicity and death. Use of specific antidote against chemical poisoning may save life, but there are very less antidotes available against chemical poisoning because these type of poisoning occur very rarely and no research is going on to develop new antidotes; these antidote are referred as orphan drugs . There is no antidote available against SM poisoning. Despite 90 years of research, there is still no

antidote for SM. This fact is especially crucial when we consider that probably at least a dozen countries have SM in their arsenals today. Development of an effective prophylactic or therapeutic antidote is an immediate requirement for the personnel particularly OPCW officials engaged in the destruction of SM. Taking into consideration increasing terrorist activities, drug development against SM is needed not only for army personnel but also for civilians. A large number of chemicals and drugs including sulphur compounds have been tested against SM or nitrogen mustard toxicity in various protocols but were found to have little or no protective effect against its systemic toxicity (2-3). There still is no effective treatment for SM toxicity and it is a challenge even today (4-5). In this review, we have summarizes the current update about the work done so far and the future strategies for the treatment for SM induced toxicity.

2. POSSIBLE MECHANISM OF SM TOXICITY

Despite 90 years of research, there is still no antidote for SM even the cytotoxic mechanisms of SM are still not understood. This fact is especially crucial when we consider that probably at least a dozen countries had SM in their arsenals today. Development of an effective prophylactic or therapeutic antidote is a requirement for the personnel particularly OPCW officials engaged in the destruction of SM. The cellular and biochemical consequences of SM exposure involve several hypotheses, which include:

- activation of Poly (ADP –Ribose) polymerase inhibitors, PARP (6) followed by alkylation of DNA (2),
- oxidative stress that induces GSH depletion leading to lipid peroxidation (7), and
- cell death and nitro-oxidative stress because of the formation of highly toxic reactant, peroxynitrite (ONOO-) that interact with Matrix

metalloproteinase, MNPs (8-9) & NF-κB (10) and promote inflammatory responses.

3. TREATMENT STRATEGY FOR SM INDUCED TOXICITY

3.1 Matrix metalloproteinase (MMPs) inhibitors

SM easily penetrates the skin and causes fluid filled blisters within hour at the dermal-epidermal junction, which is also identical pathological target for junction epidermolysis bullosa, JEB (11). JEB separates the epidermis layer from the dermis layer; it is a genetic disorder, which causes blistering of skin. Disturbance in the dermis-epidermis junction is due to the action of MMPs, which are protease causing damage to tissue and also increases the action of various activating factors during inflammatory responses and contributes to degradation of tissue (12). MMP -9 has a potential target of therapy for SM damage (13). Increase in MMP-9 correlates to increased tissue damage; therefore it is hypothesized that decrease in the MMP-9 in skin will reduce the damage of tissue after SM exposure. Studies have shown some success in the use of protease inhibitors both in vitro cell culture (14) and in an in vivo mouse model (15). Tissue inhibitors of metalloproteinase (TIMPs) are produced by many cell types in cultures and also found in body fluids and tissue extracts (16). TIMPs regulate various MMP-related degrading processes like during morphogenesis and growth (17). In SM-exposed guinea pigs, there is an imbalance in relative concentrations of MMPs and TIMPs (18). Doxycyline, an MMP inhibitor reported to inhibits the activity of both 72 – kDa MMP-2 and 92-kDa MMP-9 (19-20) but also inhibit the activity of MMP-1, MMP -8 and MMP-13 (21-22) and MMP-7 (23). The mechanism by which doxycycline causes MMP inhibition is not fully understood. Doxycycline blocks the active site of MMP molecule by binding to the associated Zn^{2+} or Ca^{2+} ions and causes conformational changes that render the proenzyme susceptible to fragmentation during (24).

3.2 Poly (ADP –Ribose) polymerase inhibitors (PARP)

Formation of apurinic sites during repair process and DNA alkylation results in the breakage of single- and double-strand DNA; which leads to the activation of PARP , a family of nuclear cell signaling enzyme involved in poly -ADP ribosylation of DNA – binding protein (25). While low levels of PARP activation signal repair excessive activity can depletes cells of NAD^+ and adenine triphosphate (ATP) resulting in cytotoxicity. Whether this results in apoptosis or necrosis depends on the cell type and other factors. The role of PARP in SM – induced cytotoxicity can be seen by using transgenic mouse model (26-27). Fibroblasts lacking PARP -1 is isolated from mice, the most abundant PARP isoform, are more likely to undergo apoptosis, when compared to wild–type cells, which prudentially undergo necrotic cell death. Unlike results in fibroblasts, immortalized keratinocytes derived from wild–type mice only exhibit apoptosis following SM treatment. These data suggest that while PARP may determine the mode of SM–induced death in some cell type, apoptosis appears to pre dominate in mouse keratinocytes. Using the HaCaT human

keratonicytes cell line it was demonstrated that SM readily stimulates PARP–1 activity and produce a dose dependent activity continuum of cell death from apoptosis to necrosis (28). Furthermore, treatment of the cell with 3 – aminobenzamide, an inhibitor of PARP, causes a discernible inhibition of necrosis. PARP inhibitors (3-(4-bromophenyl) ureidobenzamide and benzoylene urea) abrogate SM toxicity in the mouse ear vesicant model (29). These results described above suggest that PARP may contribute, atleast in part, to necrosis in keratinocytes.

3.3 Anti-inflammatory agents

3.3.1. Non-steroidal anti-inflammatory drugs (NSAIDs)

Several in vivo studies have documented increased expression of pro-inflammatory cytokines in the skin following SM exposure. Using in situ hybridization techniques, increases in interleukin (IL)-1β, IL-8, monocyte chemoattractant protein (MCP)-1, and growth related gene mRNA were noted as early as 2 h after application of liquid SM to rabbit skin (30). In mouse ear, IL-1β, IL-6, tumor necrosis factor (TNF)-α, and granulocyte monocyte-colony stimulating factor have been reported to be elevated within 6 h. Increases in IL-1α protein and IL-1β, TNF-α, macrophage inflammatory protein, MIP-2, and MCP-1 mRNA have also been detected in the dorsal skin of hairless mice after exposure to SM vapor(31-34). In cultured human keratinocytes, SM stimulates the release of IL-1β, IL-6, IL-8, and TNF-α at 100–300μM, doses relevant to in vivo exposure. Cultured skin fibroblasts have also been shown to express IL-6 in response to SM (35-36). These cytokines are thought to be key to inflammatory cell recruitment and activation at sites of injury, initiating a second phase of soluble mediator release. Cytokine expression is controlled by several signaling molecules, including the transcription factors nuclear factor-kappaB (NF-κB) (37) and activator protein-1 (AP-1) (38). NF-κB has been reported to be activated after SM exposure (39-41) and both AP-1 and NF-κB after 2-choloroethyl ethyl sulphide (CEES) exposure (42). Arachidonic acid and its cyclooxygenase and lipooxygenase products are important inflammatory mediators that have also been observed in the skin after SM exposure (43-47). Several of these mediators increase capillary permeability facilitating the influx of additional inflammatory substances including complement components, kinins, and fibrin into the dermal interstitium (46). Cyclooxygenase-2 (COX-2), the rate-limiting enzyme in prostaglandin biosynthesis, has also been identified in the epidermis of SM-treated mice (48). Findings that NAIDs reduce skin injury suggest that these mediators are important in SM toxicity (49). That COX-2 is involved in toxicity is also supported by studies showing that the extent of ear swelling and histopathological signs of lesion severity are markedly reduced in COX-2 null mice treated with SM or in wild-type mice treated with celecoxib, a COX-2–specific inhibitor (50). In contrast, loss of COX-1, the constitutive isoform of the enzyme, has no effect on cutaneous injury induced by SM. Taken together; these studies suggest an involvement of inflammatory mediators in SM cutaneous pathology. However, it

remains to be determined, which of these are important in the vesication process.

3.3.2. Glucocorticoids

In rodents, administration of betamethasone, a moderately potent anti- inflammatory glucocorticoid, from day 7 to day 14 following SM exposure, decreases airway injury, as assessed by increases in epithelial cell density, and proliferation. Treatment of animals with betamethasone for 7 days after SM also abolishes hyper-responsiveness to substance P, presumably by increasing the activity of neutral endopeptidase in airway smooth muscle (51-52). Similarly, dexamethasone, a more potent glucocorticoid analogue, administered 1 h after exposure of mice to nitrogen mustard, reduces airway inflammation, lymphocyte activity, and collagen deposition (53). Inhaled corticosteroids also improve pulmonary function in patients with chronic bronchiolitis because of SM inhalation, and this effect is synergistic with inhaled β-2 agonist bronchodilators (54). The specific inflammatory cell type and mediator involved in the pathogenic response to vesicants has not been established. Neutrophil depletion has been reported to markedly attenuate lung injury, edema, and hemorrhage after exposure of rats to CEES (55). These data, together with findings that dexamethasone blocks SM induced activation and proliferation of alveolar macrophages (56), provide support for an involvement of phagocytic leukocytes in the pathogenic response to vesicants. Newer therapeutic approaches for treating pulmonary diseases have focused on specific pro- and anti-inflammatory mediators to ameliorate vesicant-induced lung injury. For example, IFNγ, in combination with low dose prednisolone improved the lung function in patients with chronic bronchitis due to mustard gas poisoning (57). Recent observations that TNF receptor-1 knockout mice are protected from CEES-induced injury and altered lung functioning suggest that targeting TNFα may also prove effective in treating patients exposed to SM (58). Upstream signaling pathways are also promising targets for future drug development. Mechanistic studies have demonstrated activation of NF-kB and AP-1 in the lung within 1-2 h of exposure to CEES. These ubiquitous transcription factors regulate the activity of a number of inflammatory genes implicated in pulmonary toxicity, including inducible nitric oxide synthase (iNOS), COX-2, and TNFα. Mitogen activated protein kinase signaling is also upregulated in the lung following mustard exposure (59-61). Pharmacologic antagonists against one or more of these signaling molecules may prove useful in mitigating vesicant-induced pulmonary toxicity.

3.4. Inhibitors of oxidative stress

Oxidative stress is an important mechanism by which SM contributes to toxicity. Arising by a variety of mechanisms including disruption of mitochondria increases in activity of enzymes producing reactive oxygen species (ROS) and capable of redox cycling, decreases in small molecular weight intracellular antioxidants including glutathione (GSH) and various antioxidant enzymes, SM induced oxidative stress is a result of imbalances in the production and/or detoxification of ROS. Nitric oxide (NO), which has been shown to participate in SM

toxicity, likely by reacting with ROS and forming highly toxic peroxynitrite, also plays a role in oxidative stress. Increases in a variety of oxidative stress markers have been detected in tissues exposed to SM or its analogs including lipid peroxidation products, as well as protein and DNA oxidation products. Antioxidants and nitric oxide synthase inhibitors have shown varying degrees of protection against SM-induced tissue injury. Successful therapy for SM toxicity may depend on the development of new antioxidants effective against SM-induced ROS and their improved delivery to target tissues.

3.4.1. N-acetylcysteine (NAC)

Several studies have shown that GSH or the GSH prodrug, NAC, can reduce oxidative stress and toxicity induced by SM or its analogs. For example, GSH has been shown to increase the survival time of mice following inhalation of SM (62) and NAC has been shown to protect against acute lung injury induced by CEES (63-64). In a rat model, liposomes containing NAC have also been shown to protect against lung toxicity induced by CEES (55). In humans exposed to SM, NAC has also been reported to improve clinical outcomes (65).

3.4.2. Antioxidant

Quercetin, a naturally occurring bioflavonoid, by intraperitoneal injection in mice, also significantly protected the depletion of glutathione and increased the malondialdehyde level by SM. Quercetin was effective only as a pretreatment or simultaneous treatment with percutaneously applied SM (66). Further, it was reported that percutaneous administration of SM induced oxidative stress and intraperitoneal administration of gossypin (3,30,40,5,7,8-hexahydroxyflavone 8-glucoside) significantly protected against it. A very good protection was observed when gossypin was administered 30 min prior to or simultaneous to SM exposure, but not as post-treatment. Compounds that can modulate glutathione levels within the cell may reduce the cytotoxicity of SM when used as a pretreatment. L-oxothiazolidine-4-carboxylate, a cysteine precursor, increased the level of glutathione. Pretreatment of human peripheral blood lymphocytes with L-oxothiazolidine-4-carboxylate resulted in a small decrease in cytotoxicity after SM exposure. Post-treatment with L-oxothiazolidine-4-carboxylate was not beneficial (67). SM reacts with glutathione to form a glutathione–SM conjugate by the action of glutathione-S-transferase. Ethacrynic acid, an inhibitor, and oltipraz, an inducer, were ineffective in modulating this enzyme in cultured normal human epidermal keratinocytes. However, D,L-sulforaphane, a compound obtained from broccoli extract and a potent inducer of this enzyme, increased the level of this enzyme optimally. When SM was challenged with D,L-sulforphane, there was an improvement in survival compared with unpretreated SM controls (68).

3.4.3. Nitric oxide synthase (NOS) inhibitors

Nitric oxide synthase inhibitors have shown varying degrees of protection against SM-induced tissue injury. Several different arginine analogue NOS inhibitors such as L-nitroarginine methyl ester (L-NAME) have been shown to have protective activity against the toxicity of SM in primary

cultures of chick embryo neurons (69). Toxicity of SM was due to the induction or activation of NOS, thus liberating increased and toxic quantities of the reactive chemical species, NO (70-71). iNOS is responsible for the abundant NO synthesis and mainly responsible for the $ONOO^-$ production, future research is needed using highly selective iNOS inhibitors such as aminoguanidine, *S*-methylthiourea, and 1400W against mustard toxicity.

3.5. Synthetic antidotes

The synthetic aminothiol, amifostine has been extensively used as a chemical radioprotector for the normal tissues in cancer radiotherapy and chemotherapy. The cytoprotective action of amifostine is due to its conversion to free thiol metabolite (WR-1065) by the action of membrane bound alkaline phosphatse (Spencer and Goa, 1995; Capizzi, 1999; Foster and Siden, 1999). The available SM inside the body was reduced by amifostine and its analogue DRDE-07 when they were given as a prophylactic agent (Vijayaraghavan et al., 2001). The protection offered by DRDE-07 was more than 20 fold in the mouse model (Kumar et al., 2001). Prophylactic effect of DRDE-07 is better than amifostine, may be attributed to the presence of an aryl group which increases the lipophilicity and thereby the bioavailability. DRDE-07 was found to be beneficial only when SM was administered through the percutaneous route but not only by oral, subcutaneous or inhalation routes (Vijayaraghavan et al., 2004). The unusual finding with SM is that it is more toxic when administered through the percutaneous route than by oral and subcutaneous routes (Vijayaraghavan et al, 2005). It appears that DRDE-07 does not react with SM stoichiometrically, as the effective dose of DRDE-07 is comparatively high. DRDE-07 seems to be metabolised to an active compound, which in turn neutralizes or reacts with the active metabolite of SM. Few analogs of amifostine, such as DRDE-10, DRDE-21, DRDE-30, and DRDE-35 including DRDE-07, gave enormous protection in the mouse skin mode. Percutaneously administered SM significantly depleted the hepatic glutathione content and increased the percent DNA fragmentation in mice. Some of the compounds, particularly DRDE-07, DRDE-30, and DRDE- 35, significantly protected the mice after SM intoxication. The histopathological lesions in liver and spleen induced by percutaneously administered SM were also reduced by pretreatment with these compounds (Kulkarni et al., 2006). These classes of compounds, though giving very good protection against SM but failing to give appreciable protection against 2-chloroethyl ethyl sulphide and nitrogen mustard, showed the mechanism and toxicity of the mustard agents are different. These compounds may prove effective as prototypes for the designing of more successful prophylactic drugs for SM and nitrogen mustard.

4. CONCLUSION

All molecular mechanisms of cytotoxicity must take into account in order to be effective, a protective treatment against SM. Therefore, it would be interesting to combine several individual potent agents, each blocking one of the toxic mechanisms induced by mustards. Thus, a combination of cell membrane receptor blockers,

antioxidants, NOS inhibitors, peroxynitrite scavengers, PARP inhibitors, and synthetic antidotes must be investigated.

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