Antioxidants Countermeasures Against Sulfur Mustard

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Abstract: Sulfur mustard (SM) is a vesicant chemical warfare agent that persists as a serious menace from the viewpoint of acute and chronic toxicity, simple synthesis and no effective treatment currently being available. The two most deleterious basic molecular mechanisms in SM poisoning are: inflammation and over-activation of poly(ADP-ribose) polymerase and the resulting DNA alkylation. Oxidative stress is the common consequence of these pathway activations. In the present review, the significance of oxidative stress in SM poisoning is discussed along with research on antioxidant therapy as a suitable antidote. The temporal dynamics of the redox imbalance, the antioxidant depletion and impact this has on tissues are described as the pathologies induced by SM. Special attention is paid to ameliorating the damage using low molecular weight antioxidants. Melatonin, epigallocatechin gallate and flavone derivatives, in particular, have been studied in recent experiments. The suitability of these antioxidants for therapy purposes is considered in a separate chapter. The review concludes with a view to the future and the studies needed on antioxidant therapy as a countermeasure to SM poisoning.

Keywords: apoptosis, DNA repair, inflammation, oxidative stress, poly(ADP-ribose) polymerase, sulfur mustard.

INTRODUCTION

Sulfur mustard (SM), bis(2-chloroethyl)sulfide (CAS: 505-60-2), is a well known chemical warfare agent known since the beginning of industrial chemical warfare.Itwas used for military purposes by several countries during the 20th century. The battlefields of World War I and misuseof chemical warfare in the Iran-Iraq war (1980-1988) can be mentioned as examples [1]. SM is a cytotoxic agent. From a military point of view, it is classified as a vesicant chemical agent as exposure manifests as blisters. Some other toxins are also cytotoxic with a similar toxicity mechanism and manifestation as SM. Nitrogen mustards and glutathione half mustard 1,2-dibromoethane as well as some other dihalogenide compounds forming GSH episulfonium electrophiles for example [2].

However the toxicity mechanism of SM is not well-understood. Scientific investigation is complicated by the fact that blisters are not formed in furry laboratory animals as their skin is not as keratinized as humans [3]. The seriousness of SM exposure is underlined by the fact that there is no clinically- proven antidotal therapy. This is unlike other highly toxic chemical warfare nerve agents that can be treated by commercially available antidotes [4].

Though SM is designated as vesicant, exposure is typically more serious than implied by the name. SM is an alkylating reagent with wide substrate specificity. Its presence in the organism leads to modification of macromolecules including DNA and other nuclear components [5]. Oxidative stress is also significantly implicated in SM toxicity [6]. Commonly known blisters are only a symptomatic manifestation of less importance than the destabilizing of genetic information. The present review focuses on antioxidants as a countermeasure to SM exposure with reference to the most relevant facts, the limitations and molecular mechanisms involved.

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SULFUR MUSTARD DAMAGE FROM A MEDICAL POINT OF VIEW

SM exposure typically leads to skin injury. Owing to its hydrophobicity, it accumulated in lipids and then skin or lung penetration. After absorption, SM is distributed throughout the body resulting in severe damage to liver, kidney, spleen and other organs [7]. Brain damage and decline in cognitive function is also a serious complication after poisoning by sulfur mustard [8]. As witnessed in Iranian veterans from the Iran-Iraq war, the most affected sites are lungs, peripheral nerves, skin (including head and neck) and eyes when sulfur mustard is used as a chemical weapon [9]. The skin irritation is based on chemical modification of the skin and linked with the creation of blisters in humans. Collagen is also seriously damaged by SM. Recent experiments have found an inability of collagenase to degrade the SM modified collagen despite increases in collagenase level [10].

SM is not a highly toxic compound from the viewpoint of median lethal dose (LD_{50}) . E.g. the LD_{50} for subcutaneously administered SM is 23 mg/kg for mice [11]. Compared to SM nerve agents, another group of chemical warfare agents are the more toxic. E.g. the LD_{50} for sarin administered subcutaneously into mice is 1.35 μmol/kg (i.e. 189 μg/kg) with marked mortality for up to 24 hours [12]. The neurotoxin sarin is thus more than 100Xmore toxic than SM. On the other hand, SM causes lasting adverse effects involving complex molecular mechanisms as will be

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discussed and the mechanism of its toxicity is unlike that of other chemical warfare agents.

Although, there is no firm evidence of an increased incidence of cancer after accidental exposure to SM, the detrimental effects of chronic exposure are well-documented [13]. Most of the clinical examinations have been done on Iranian victims of the Iraqi chemical warfare attacks in the 1980s. Hence many adverse effects of SM in humans have only been recognized by Iranian researchers. Rovell *et al*. [14] described reversal of the skin changes. On the other hand, they found progressive deterioration of the eyes/retina as keratitis. Bronchiolitis obliterans referred to as "mustard lung" was diagnosed as well. The psychological, immunological and reproductive effects of SM exposure are also considered by these authors [14]. Well-recognised exposure to SM is by skin blisters. Surprisingly, the skin damage, despite its visibly "horrible" appearance, is not typically malignant. Hyperpigmentation, dry skin, multiple cherry angiomas, atrophy and hypopigmentation in forty Iranian male subjects 16 to 20 years after poisoning in warfare events were reported by Hefazi *et al*. [15].

INFLAMMATION

Extensive inflammation accompanies SM exposure as many novel epitopes arise. The effects on the immune system have been clearly recognized from both animal models and human victims. The recovery of the immune system after SM poisoning remains unclear [16]. The inflammatory response begins shortly after exposure. The damaged regions produce inflammation markers such as cyclooxygenase 2 (COX 2), tumor necrosis factor α (TNF α) and inducible nitric oxide synthase (iNOS) as reported by Malaviya *et al*. for a rat model [17]. The authors also described the generation of cytotoxic inflammatory proteins and discuss the contribution of inflammation to the pathogenic processes. However, the role of iNOS is not clear as the other scientists have reported decreased iNOS expression after SM analogue, 2-chloroethyl ethyl sulfide (CEES), in murine macrophages [18]. Apart from cytokine production, TNF α positive neutrophils were discovered circulating in the blood of exposed animals and gradually infiltrated adjacent tissues [19]. Similar findings were reported by Mishra *et al.* for a guinea pig model [20]. These authors found infiltration of CD4+ and CD8+ T cells into the affected tissues. Given the relevance of lymphocytes the prominent role of T cells in SM induced inflammation was recognized by Ekstrand-Hammarstrom *et al*. [21]. The inflammation is also clear from cell lines experiments. A human cell model confirmed the production of inflammatory cytokines such as interleukin (IL) 6 in the presence of SM [22]. The model also confirmed dose-dependent cytotoxic effects. The idea that suppression of inflammation could be beneficial was proposed by Wormser *et al*. [23]. The authors found reduced epidermal necrosis, acute inflammation, and hemorrhage in COX 2 deficient mice. COX 1 deficient mice and wild type ones had typical signs of poisoning. The detrimental effects of inflammation are also discussed by other authors [24]. Use of anti-inflammatory drugs is the method of choice in reducing the impact of SM on the body. Wormser *et al*. found a reduction in acute inflammation, epidermal ulceration and necrosis following SM poisoning when a povidone-iodine preparation containing steroidal and non-steriodal anti-inflammatory drugs was administered [25]. In another experiment, restoration of neutrophil induced inflammation with consequent oxidative insult was achieved using anti - TNF α antibody therapy [19].

However, the link between the immune system and SM toxicity is complex. SM induces neutropenia and this increases the susceptibility of SM-exposed individuals to infection due to SM cytostatic action [26]. The combination of neutropenia with recommendations to pharmacologically suppress inflammation is noteworthy. Increased susceptibility to infectious diseases of exposed subjects might be fatal in the case of infected wounds. From this point of view, macrolide antibiotics have a dual positive action [27, 28]. Firstly, they can protect the body as a standard antibiotic. Secondly, the SM caused overproduction of pro-inflammatory cytokines such as IL 1, IL 6, IL 8 and TNF α is inhibited.

DNA DAMAGE AND POLY(ADP-RIBOSE) POLYMERASE ACTIVATION

SM is a highly reactive compound. The reactivity is non-specific with no predilection for DNA alone. However, chemical modification of DNA is more damaging than modification of other molecular targets. Apart from simple alkylation, cross linking of macromolecules is accomplished as a result of SM reactions within the organism. The chemical reactivity is well-understood. In aqueous conditions, the chloride anion is split from the ethyl moiety in a first order reaction mechanism. Unstable and positively charged episuflonium is the reaction product [29]. The episulfonium is a strong electrophile which attacks many target structures. 7-(2-hydroxyethylthioethyl) guanine was the major adduct experimentally identified using HPLC [30]. The DNA can be cross linked as the SM molecule contains two reactive sites. The first adduct on guanine, 7-(2-chloroethylthioethyl) guanine, is still reactive. Next to the 7-(2-hydroxyethylthioethyl) adduct on guanine, the dimeric guanine, di-(2-guanin-7-yl-ethyl) sulfide, is a product of the degraded DNA [31]. The SM-guanine adducts are formed fast without any transition state [32]. The mechanism of SM alkylation of guanine is depicted in Fig. (**1**).

The alkylated nucleotides are excised from the DNA double strand. Two pathways are associated with the DNA protection: nucleotide excision repair and base excision repair [33]. Homologous recombination DNA repair is necessary to suppress SM toxicity. Cells lacking the recombination DNA repair are significantly more sensitive to SM poisoning [34]. SM is able to induce single or double strand breaks in a dose response manner. E.g. Mol *et al*. scored frequency of single strand breaks as 0.05 per 10^9 Da DNA for cells kept in 1 μmol/l SM [35]. Poly(ADP-ribose) polymerase (PARP) is activated as a result of the DNA damage. This step appears to be crucial in SM toxicity. Activation of PARP is necessary for DNA repair and triggering of apoptosis. However, excessive activation of PARP leads to subsequent depletion of the NAD and ATP cell pool [36]. Kehe *et al*. described the preference for apoptosis over necrosis in HaCaT cell lines exposed to 10 - 1,000 μmol/l SM when a PARP inhibitor, 3-aminoben-

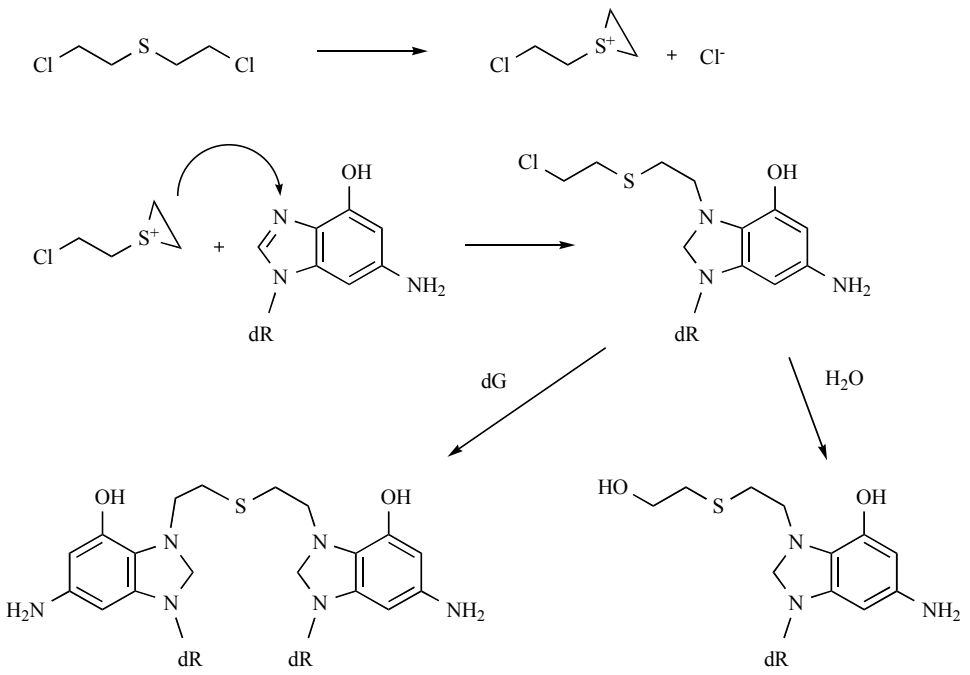


Fig. (1). Sulfur mustard alkylation of 2-deoxyguanosine (dG) in aqueous conditions; dashed arrow: electrophilic attack; dR - deoxyribose. Mono alkylated guanine adduct and dimeric guanine adduct are the reaction products. The reported mechanism is in compliance with the mentioned references [31, 32].

zamide, was present in the medium [37]. Both apoptosis and necrosis may be linked to PARP. However, the energy demanding apoptosis cannot be completed when ATP is exhausted due to PARP hyperactivity. The initiated apoptosis then terminates as necrosis [38].

OXIDATIVE STRESS

Two pathologies caused by SM are linked to oxidative stress. The first relates to inflammation and the second to DNA damage. Unfortunately, experiments aimed at investigating of oxidative stress in SM toxicity do not provide unambiguous conclusions. Evaluating the role of oxidative stress role is confounded as there is no universal agreement on the role of oxidative stress under physiological and under pathophysiological conditions [39]. Oxidative stress also has unequal effects in different ontogenesis phases and under individual conditions [40]. Nevertheless, the basic links between SM and oxidative stress have been recognized and independently confirmed by several researchers.

The balance between reactive oxygen species (ROS) and antioxidant levels is altered in SM poisoned subjects. However, the oxidative stress is probably tissue specific as plasma antioxidants can be recovered in one day after exposure [41]. Unequal depletion of individual low molecular weight antioxidants can be expected. As discussed above, acute inflammation is tightly associated with oxidative insult. Activated neutrophils participate in oxidative bursts within a few hours after SM exposure and this can be suppressed by an anti-inflammatory therapy [19]. The effect of the immune system on oxidative stress is not surprising and peculiar to chemical toxins such as the SM. Similar processes have been described during acute phases of infectious disease) [42]. The second reason for the generation of ROS is based on cellular depletion of energy due to over-stimulation of PARP and consequent lack of cellular antioxidants [43]. For this reason, PARP inhibitors are recommended for the scientific investigation of novel antidotes against SM / nitrogen mustards [44].

Oxidative stress after SM exposure has been independently reported by several researchers. Pal *et al.* investigated oxidative stress markers in hairless mice after exposure to SM analogue, CEES. The mice were exposed for 12 hours and CEES was administered on the dorsal skin [45]. The mice were sacrificed at time intervals 3, 6, 9, 12, 24, 48, 72, and 168 hours. The authors found a significantly elevated marker of DNA oxidation, 8-oxo-2-deoxyguanosine, in skin tissue from six hours to one day after sacrifice. A marker of lipid peroxidation, 4-hydroxynonenal, was assessed as a protein adduct. It was elevated for the whole time interval up to 168 hours. In another study, reactive nitrogen species (RNS) were indirectly confirmed in human keratinocytes (HaCaT) cell lines in the presence of 100 or 300 μmol/l of SM for a 1-6 hour time interval [46]. Cell lines experiments confirm time and SM concentration dependent expression of both iNOS and eNOS. Human bronchial epithelial cells and CEES were used as a model by Gould *et al*. [47]. They analyzed mitochondrial ROS using the probe MitoSOX. Cells exposed to 900 μmol/l of CEES were analyzed for ROS at time intervals 2, 4, 6, 8, 12, 24, and 48 hours. Mitochondrial ROS increased up 12 hours. After that, it decreased. When compared to controls, time intervals 12 and 24 hours showed significantly increased ROS equal to double the control values. A stable marker of general oxidative imbalance, total level of carbonylated plasma proteins, was assessed in Wistar rats exposed to SM in

Table 1. Alteration in Oxidative Stress Markers After Sulfur Mustard Exposure

Marker	Time post exposure	Experimental model	Reference
Elevated 8-oxo-2-deoxyguanosine	6-24 hours	skin tissue of hairless mice exposed to 2-chloroethyl ethyl sulfide	[45]
Elevated 4-hydroxynonenal	3-168 hours		
Expression of iNOS and eNOS	1-6 hours	human keratinocytes HaCaT exposed to sulfur mustard	[46]
Mitochondrial reactive oxygen species	12-24 hours	human bronchial epithelial cells exposed to 2-chloroethyl ethyl sulfide	[47]
Elevated level of carbonylated plasma proteins	2 hours	Wistar rats intradermally exposed to sulfur mustard	[48]

selected doses 5 - 80 mg/kg [48]. The animals were sacrificed two hours after exposure. Those exposed to 20 and 80 mg/kg of SM had twice the plasma protein carbonyl levels of the controls. The oxidative stress reported in these experiments is summarized in Table **1**.

Owing to this research, it is now known that SM participates in the generation of oxidative stress several hours post exposure. The long time interval needed for oxidative imbalance is important for treatment efficacy. Compared to e.g. exposure to nerve agents, where treatment must be initiated immediately after exposure [49], the postponed oxidative stress in the case of SM is a good opportunity to start treatment in a specialized institution without risk of delay. The suitability of antioxidants to counteract the oxidative stress and ameliorate SM caused pathology is discussed in the next chapter.

ANTIOXIDANTS IN SULFUR MUSTARD POISONING

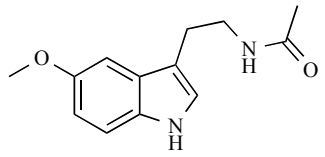
The mitigating effect of antioxidant therapy has been experimentally confirmed by a number of authors. Individual antioxidants are taken for suitable antidotes [50]. The underlying hypothesis for the use of antioxidants stems from the fact that low molecular weight antioxidants are significantly depleted after SM poisoning. Administration of exogenous antioxidants can offset this situation. The role of enzymatic antioxidants apart from low molecular weight antioxidants should also be considered. Shohrati *et al*. reported elevated catalase activity in the case of nearly 50 % of the lungs of victims exposed to SM in 1987. The alterations were still found 20 years after the incidental poisoning in the Iran - Iraq war [51]. However, the victims of the same war events had significantly decreased reduced glutathione (GSH) in serum. The decrease was estimated to be 40 % lower than healthy controls [52]. The mechanism of the low molecular weight antioxidant depletions however is not clear. In a Wistar rat model, dermal exposure to SM in both doses 20 and 80 mg/kg led to exhaustion of liver GSH levels from 707 in controls to 379 respectively 322 nmol/g in the poisoned animals [53]. However, the total level of low molecular weight antioxidants represented as ferric reducing antioxidant power (FRAP) was steady in the plasma, blood, and liver and no significant changes were found for either SM dose. This could imply that some antioxidants are substituted by others. An experiment performed by Jafari *et al.* showed a dose dependent depletion of GSH level in liver and brain of Wistar rats i.p. poisoned with SM 1 - 80 mg/kg [54]. Despite the unanswered questions about SM pathology, depletion of endogenous antioxidants and the link to oxidative stress are reasonably proven in both animals and humans. Despite the relatively long time period between the Iran-Iraq war and actual research, the results from animal model correspond well to the recognized pathologies in human victims.

Application of exogenous low molecular weight antioxidants is experimentally proving suitable for SM induced pathology by some researchers. Unfortunately, no comprehensive study that compares individual low molecular weight antioxidant efficacy has been done to date. The mitigating effects of antioxidants have been reported in animals exposed to sulfur mustard. The animals had significantly reduced markers of the oxidative stress malondialdehyde and carbonylated proteins, and increased plasma FRAP values when treated with melatonin [48].

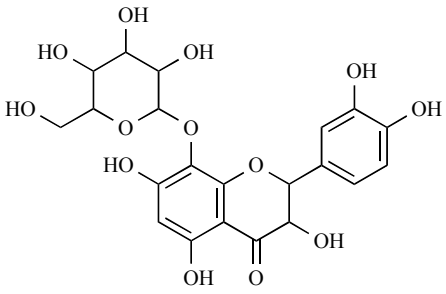
Table 2. Depletion of Low Molecular Weight Antioxidants as a Consequence of Sulfur Mustard Exposure

Antioxidant	Decrease to controls	Time post exposure	Exposure/model	Reference
serum GSH	40 %	20 years	human victims of the Iran - Iraq war	[52]
GSH in liver	~ 45 %	24 hours	Wistar rat, dermal exposure to sulfur mustard	[53]
FRAP in plasma, blood, and liver	none			
GSH in liver	up 30 %	48 hours	Wistar rat, intraperitoneal exposure to sulfur mustard	[54]
GSH in brain	up 21 %			

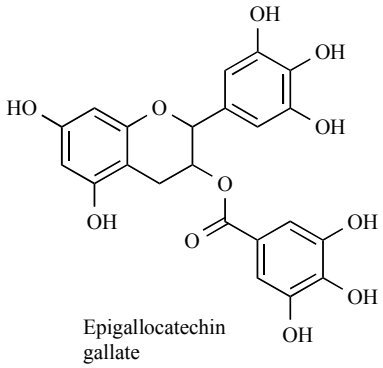
Compared to melatonin, epigallocatechin gallate (EGCG) administered prior to SM had only limited effects on redox homeostasis when oxidative stress markers were assessed one day after SM exposure [53]. EGCG applied in doses of 10 and 20 mg/kg did not improve low molecular weight antioxidant levels and did not suppress oxidative stress. On the other hand, EGCG had significant effects on glutathione reductase levels and down regulated the activity of caspase 3 indicating suppression of apoptosis. Application of low molecular weight antioxidants to laboratory animals shows relatively quite high protective indices. Flavone extract from *Hippophae rhamnoides* had a protective index 1.7 - 2.4 against mustard gases [55]. The extract was not, unfortunately, precisely characterized limiting the reproducibility of the results. Chemically defined flavone, gossypin (3,3',4',5,7,8-hexahydroxyflavone 8-glucoside), mitigated SM detrimental impact on mice with a high protective index scored as 4.8 [56]. The chemical structures of the tested antioxidants are depicted below (Fig. **2**).



Melatonin



Gossypin



Epigallocatechin gallate

Fig. (2). Low molecular weight antioxidants tested as prophylaxis against SM exposure [48, 53, 56].

FUTURE TRENDS AND EXPECTATIONS

It should be emphasized that the expected costs of therapy based on low-molecular weight antioxidants are quite low and there are minimal adverse effects in course of the therapy. Unfortunately, a comprehensive study comparing individual low molecular weight antioxidants for SM induced pathogenesis treatment has not been done. As seen in the discussion, administration of antioxidants can ameliorate oxidative stress; however, antioxidants such as EGCG have more apoptosis regulating effects than direct impact on oxidative insult. Individual antioxidants have also unequal ability to penetrate tissues and protect lipid membranes from radical degradation. E.g. melatonin penetrates the blood brain barrier and protects the central nervous system; moreover, it can facilitate protection of cell and mitochondrial membranes [57]. Ascorbic acid, another common antioxidant, has a completely different fate in body as it is hydrophilic, well-absorbed, and simply excreted [58]. Vitamin E, a wide group of tocopherol and tocotrienols, is a lipophilic antioxidant deponable in lipid membranes where it can quench lipid peroxidation. It has been recognized to be effective in reducing poisoning by nitrogen mustard [59]. Similar effects can be expected for exposure to sulfur mustard. Some expectations for SM therapy can be also given when considered the current research of radioprotectives. Both thiol and/or selenium containing antoxidants are suitable for countermeasure against ionizing radiation. E.g. GSH is more suitable as a radioprotective than N-acetyl cysteine and thioproline [60]. Monoselenides selenomethionine, methylselenocysteine and diselenides selenocystine, selenopropionic acid were examined *in vitro* and the selenopropionic acid was proved to be the most effective [61]. It can be inferred that the mentioned compounds will be effective for SM therapy as well and the next research will use antioxidants being tested in another consequences.

Owing to the discussed facts, the probability that SM poisoning will be treated with a new antidote with high efficacy is very low. On the other hand, a combination of anti-inflammatory treatment with oxidative imbalance therapy may be effective in significantly reducing the detrimental effects of SM.

CONFLICT OF INTEREST

None declared.

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REFERENCES

[1] Evison, D.; Hincley, D.; Rice, P. Regular review: chemical weapons, *Brit. Med. J.*, **2002**, *324*, 332-335

[2] Guengerich, F.P. Activations of dihaloalkanes by thiol-dependent mechanisms. *J. Biochem. Mol. Biol.*, **2003**, *36*, 20-27.

[3] Paromov, V.; Suntres, Z.; Smith, M.; Stone, W.L. Sulfur mustard toxicity following dermal exposure: role of oxidative stress, and antioxidant therapy, *J. Burns Wounds*, **2007**, *7*, 60-85

[4] Pohanka, M.; Musilek, K.; Kuca, K. Progress of biosensors based on cholinesterase inhibition. *Curr. Med. Chem*., **2009**, *16*, 1790-1798.

[5] Lodhi, I.J.; Sweeney, J.F.; Clift, R.E.; Hinshaw, D.B. Nuclear dependence of sulfur mustard-mediated cell death. *Toxicol. Appl. Pharmacol.*, **2001**, *170*, 69-77.

[6] Laskin, J.D.; Black, A.T.; Jan, Y.H.; Sinko, P.J.; Heindel, N.D.; Sunil, V.; Heck, D.E.; Laskin, D.L. Oxidants and antioxidants in sulfur mustard-induced injury. *Ann. N.Y. Acad. Sci.*, **2010**, *1203*, 92-100.

[7] Lomash, V.; Deb, U.; Rai, R.; Jadhav, S.E.; Vijayaraghavan, R.; Pant, S.C. Designing of mouse model: a new approach for studying sulfur mustard-induced skin lesions. *Burns*, **2011**, In press.

[8] Sharma, D.R.; Sunkaria, A.; Bal, A.; Bhutia, Y.D.; Vijayaraghavan, R.; Flora, S.J.; Gill, K.D. Neurobehavioral impairments, generation of oxidative stress and release of pro-apoptotic factors after chronic exposure to sulfur mustard in mouse brain. *Toxicol. Appl. Pharmacol.*, **2009**, *240*, 208-218.

[9] Zojaji, R.; Balali-Mood, M.; Mirzadeh, M.; Saffari, A.; Maleki, M. Delayed head and neck complications of sulphur mustard poisoning in Iranian veterans. *J. Laryngol. Otol.*, **2009**, *123*, 1150-1154.

[10] Naderi, M.; Jadidi, K.; Falahati, F.; Alavi, S.A. The effect of sulfur mustard and nitrogen mustard on corneal collagen degradation induced by the enzyme collagenase. *Cutan. Ocul. Toxicol.*, **2010**, *29*, 234-240.

[11] Vijayaraghavan, R.; Kulkami, A.; Pant, S.C.; Kumar, P.; Rao, P.V.; Gupta, N.; Gutam, A.; Ganesan, K. Differential toxicity of sulfur mustard administered through percutaneous, subcutaneous, and oral routes. *Toxicol. Appl. Pharmacol.*, **2005**, *202*, 180-188.

[12] Clement, J.G. Toxicity of the combined nerve agents GB/GF in mice: efficacy of atropine and various oximes as antidotes. *Arch. Toxicol.*, **1994**, *68*, 64-66.

[13] Ghanei, M.; Harandi, A.A. Lung carcinogenicity of sulfur mustard. *Clin. Lung Cancer.*, **2010**, *11*, 13-17

[14] Rowell, M.; Kehe, K.; Balszuweit, F.; Thiermann, H. The chronic effects of sulfur mustard exposure. *Toxicology*, **2009**, *263*, 9-11.

[15] Hefazi, M.; Maleki, M.; Mahmoudi, M.; Tabatabaee, A.; Balali-Mood, M. Delayed complication of sulfur mustard poisoning in the skin and the immune system of Iranian veterans 16-20 years after exposure. *Int. J. Dermatol.*, **2006**, *45*, 1025-1031.

[16] Kehe, K.; Balszuweit, F.; Steinritz, D.; Thiermann, H. Molecular toxicology of sulfur mustard-induced cutaneous inflammation and blistering. *Toxicology*, **2009**, *263*, 12-19.

[17] Malaviya, R.; Sunil, V.R.; Cervelli, J.; Anderson, D.R.; Holmes, W.W.; Conti, M.L.; Gordon, R.E.; Laskin, J.D.; Laskin, D.L. Inflammatory effects of inhaled sulfur mustard in rat lung. *Toxicol. Appl. Pharmacol.*, **2010**, *248*, 89-99.

[18] Qui, M.; Paromov, V.M.; Yang, H.; Smith, M.; Stone, W.L. Inhibition of inducible nitric oxide synthase by a mustard gas analog in murine macrophages. *BMC Cell Biol.*, **2006**, *7*, 39.

[19] Wormser, U.; Brodsky, B.; Proscura, E.; Foley, J.F.; Jones, T.; Nyska, A. Involvement of tumor necrosis factor-alpha in sulfur mustard-induced skin lesion; effect of topical iodine. *Arch. Toxicol.*, **2005**, *79*, 660-670.

[20] Mishra, N.C.; Rir-sima-ah, J.; March, T.; Weber, W.; Benson, J.; Jaramillo, R.; Seagrave, J.C.; Schultz, G.; Grotendorst, G.; Sopori, M. Sulfur mustard induces immune sensitization in hairless guinea pigs. *Int. Immunopharmacol.*, **2010**, *10*, 193-199.

[21] Ekstrand-Hammarstrom, B.; Wigenstam, E.; Wigenstam, E.; Bucht, A. Inhalation of alkylating mustard causes long-term T cell-dependent inflammation in airways and growth of connective tissue. *Toxicology*, **2011**, *280*, 88-97.

[22] Emmler, J.; Hermanns, M.I.; Steinritz, D.; Kreppel, H.; Kirkpatrick, C.J.; Bloch, W.; Szinicz, L.; Kehe, K. 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.*, **2007**, *19*, 657-665.

[23] Wormser, U.; Langenbach, R.; Peddada, S.; Sintov, A.; Brodsky, B.; Nyska, A. Reduced sulfur mustard-induced skin toxicity in cyclooxygenase-2 knockout and celecoxib-treated mice. *Toxicol. Appl. Pharmacol.*, **2004**, *200*, 40-47.

[24] Amitai, G.; Adani, R.; Fishbein, E; Meshulam, H.; Laish, I.; Dachir, S. Bifunctional compounds eliciting anti-inflammatory and anti-cholinesterase activity as potential treatment of nerve and blister chemical agents poisoning. *Chem. Biol. Interact.*, **2005**, *157*, 361-362.

[25] Wormser, U.; Sintov, A.; Brodsky, B.; Casillas, R.P.; Nyska, A. Protective effect of topical iodine containing anti-inflammatory drugs against sulfur mustard-induced skin lesions. *Arch. Toxicol.*, **2004**, *78*, 156-166.

[26] Anderson, D.R.; Holmes, W.W.; Lee, R.B.; Dalal, S.J.; Hurst, C.G.; Maliner, B.I.; Newmark, J.; Smith, W.J. Sulfur mustard-induced neutropenia: treatment with granulocyte colony-stimulating factor. *Mil. Med.*, **2006**, *171*, 448-453.

[27] Gao, X.; Ray, R.; Xiao, Y.; Barker, P.E.; Ray, P. Inhibition of sulfur mustard-induced cytotoxicity and inflammation by the macrolide antibiotic roxithromycin in human respiratory epithelial cells. *BMC Cell Biol.*, **2007**, *8*, 17.

[28] Gao, X.; Anderson, D.R.; Brown, A.W.; Lin, H.; Amnuaysirikul, J.; Chua, A.L.; Holmes, W.W.; Ray, P. Pathological studies on the protective effect of a macrolide antibiotic, roxithromycin, against sulfur mustard inhalation toxicity in a rat model. *Toxicol. Pathol.*, **2011**, *39*, 1056-1064.

[29] Hamza, A.; Broch, H.; Vasilescu, D. Quantum molecular study of S-methylated forms of sulfur mustard. *Chem. Phys.*, **1996**, *204*, 373-389.

[30] Ludlum, D.B.; Austin-Ritchie, P.; Hagopian, M.; Niu, T.Q.; Yu, D. Detection of sulfur mustard-induced DNA modifications. *Chem. Biol. Interact.*, **1994**, *91*, 39-49.

[31] Matijasevic, Z.; Stering, A.; Niu, T.Q.; Austin-Ritchie, P.; Ludlum, D.B. Release of sulfur mustard-modified DNA bases by Escherichia coli 3-methyladenine DNA glycosylase II. *Carcinogenesis*, **1996**, *17*, 2249-2252.

[32] Broch, H.; Hamza, A.; Vasilescu, D. Ab initio modeling of the interaction between guanin-cytosine base pair and mustard alkylating agents. *Int. J. Quant. Chem.*, **1996**, *60*, 1745-1764.

[33] Jowsey, P.A.; Williams, F.M.; Blain, P.G. DNA damage, signaling and repair after exposure of cells to the sulphur mustard analogue 2-chloroethyl ethyl sulphide. *Toxicology*, **2009**, *257*, 105-112.

[34] Jowsey, P.A.; Williams, F.M.; Blain, P.G. The role of homologous recombination in the cellular response to sulphur mustard. *Toxicol. Lett.*, **2010**, *197*, 12-18.

[35] Mol, M.A.; van der Schans, G.P.; Lohman, P.H. Quantification of sulfur mustard-induced DNA interstrand cross-links and single-strand breaks in cultured human epidermal keratinocytes. *Mutat. Res.*, **1993**, *294*, 235-245.

[36] Hinshaw, D.B.; Lodhi, I.J.; Hurley, L.L.; Atkins, K.B.; Dabrowska, M.I. Activation of poly [ADP-ribose] polymerase in endothelial cells and keratinocytes: role in an *in vitro* model of sulfur mustard-mediated vesication. *Toxicol. Appl. Pharmacol.*, **1999**, *156*, 17-29.

[37] Kehe, K.; Raithel, K.; Kreppel, H.; Jochum, M.; Worek, F.; Thiermann, H. Inhibition of poly(DP-ribose) polymerase (PARP) influences the mode of sulfur mustard (SM)-induced cell death in HaCaT cells. *Arch. Toxicol.*, **2008**, *82*, 461-470.

[38] Cole, K.K.; Perez-Polo, J.R. Poly(ADP-ribose) polymerase inhibition prevents both apoptotic-like delayed neuronal death and necrosis after H_2O_2 injury. *J. Neurochem.*, **2002**, *82*, 19-29.

[39] Juranek, I.; Bezek, S. Controversy of free radical hypothesis: reactive oxygen species - cause or consequence of tissue injury? *Gen. Physiol. Biophys.*, **2005**, *24*, 263-278.

[40] Bezek, S.; Ujhazy, E.; Dubovicky, M.; Mach, M. Nongenomic memory of foetal history in chronic diseases development. *Neuroendocrinol. Lett.*, **2008**, *29*, 620-626.

[41] Pohanka, M.; Stetina, R. Shift of oxidants and antioxidants levels in rats as a reaction to exposure to sulfur mustard. *J. Appl. Toxicol.*, **2009**, *29*, 643-647.

[42] Bandouchova, H.; Pohanka, M.; Vlckova, K.; Damkova, V.; Peckova, L.; Sedlackova, J.; Treml, F.; Vitula, F.; Pikula, J. Biochemical responses and oxidative stress in Francisella tularensis infection: a European brown hare model. *Acta Vet. Scand.*, **2011**, *53*, 2.

[43] Korkmaz, A.; Yaren, H.; Kunak, Z.; Uysal, B.; Kurt, B.; Topal, T.; Kenar, L.; Ucar, E.; Oter, S. Epigenetic perturbations in the pathogenesis of mustard toxicity; hypothesis and preliminary results. *Interdiscip. Toxicol.*, **2008**, *1*, 236-241.

[44] Korkmaz, A.; Yaren, H.; Topal, T.; Oter, S. Molecular targets against mustard toxicity: implication of cell surface receptors, peroxynitrite production, and PARP activation. *Arch. Toxicol.*, **2006**, *80*, 662-670.

[45] Pal, A.; Tewari-Singh, N.; Gu, M.; Agarwal, C.; Huang, J.; Day, B.J.; White, C.W.; Agarwal, R. Sulfur mustard analog induces oxidative stress and activates signaling cascades in the skin of SKH-1 haireless mice. *Free Radic. Biol. Med.*, **2009**, *47*, 1640-1651.

[46] Steinritz, D.; Elischer, A.; Balszuweit, F.; Gonder, S.; Heinrich, A.; Bloch, W.; Thiermann, H.; Kehe, K. Sulphur mustard induces time- and concentration dependent regulation of NO-synthesizing enzymes. *Toxciol. Lett.*, **2009**, *188*, 263-269.

[47] Gould, N.S.; White, C.W.; Day, B.J. 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.*, **2009**, *328*, 732-739.

[48] Pohanka, M.; Sobotka, J.; Jilkova, M.; Stetina, R. Oxidative stress after sulfur mustard intoxication and its reduction by melatonin: efficacy of antioxidant therapy during serious intoxication. *Drug Chem. Toxicol.*, **2011**, *34*, 85-91.

[49] Pohanka, M.; Sobotka, J.; Svobodova, H.; Stetina, R. Investigation of oxidative stress in blood, brain, kidney, and liver after oxidme antidote HI-6 application in a mouse experimental model. *Drug Chem. Toxciol.*, **2011**, *34*, 255-260.

[50] Korkamaz, A.; Kunak, Z.I.; Paredes, S.D.; Yaren, H.; Tan, D.X.; Reiter, R.J. The use of melatonin to combat mustard toxicity. Review. *Neuroendocrinol. Lett.*, **2008**, *29*, 614-619.

[51] Shohrati, M.; Ganei, M.; Shamspour, N.; Jafari, M. Activity and function in lung injuries due to sulphur mustard. *Biomarkers*, **2008**, *13*, 728-733.

[52] Shohrati, M.; Ghanei, M.; Shamspour, N.; Babaei, F.; Abadi, M.N.; Jafari, M.; Harandi, A.A. Glutathione and malondialdehyde levels in late pulmonary complications of sulfur mustard intoxication. *Lung*, **2009**, *188*, 77-83.

[53] Pohanka, M.; Sobotka, J.; Stetina, R. Sulfur mustard induced oxidative stress and its alteration by epigallocatechin gallate. *Toxicol. Lett*., **2011**, *201*, 105-109.

[54] Jafari, M. Dose- and time- dependent effects of sulfur mustard on antioxidant system in liver and brain of rat. *Toxicology*, **2007**, *231*, 30-39.

[55] Vijayaraghavan, R.; Gautam, A.; Kumar, O.; Pant, S.C.; Sharma, M.; Singh, S.; Kumar, H.T.; Singh, A.K.; Nivsarkar, M.; Kaushik, M.P.; Sawhney, R.C.; Chaurasia, O.P.; Prasad, G.B. Protetive effect of ethanolic and water extracts of sea buckthorn (Hippophae rhamnoides L.) against the toxic effects of mustard gas. *Indian J. Exp. Biol.*, **2006**, *44*, 821-831.

[56] Gautam, A.; Vijayaraghavan, R. Prophylactic effect of gossypin against percutaneously administered sulfur mustard. *Biomed. Environ. Sci.*, **2007**, *20*, 250-259.

[57] Pohanka, M. Alzehimer´s disease and related neurodegenerative disorders: implication and counteracting of melatonin. *J. Appl. Biomed.*, **2011**, *196*, 185-196.

[58] Padayatty, S.J.; Katz, A.; Wang, Y.; Eck, P.; Kwon, O.; Lee, J.H.; Chen S.; Corpe, C.; Dutta, A.; Dutta, S.K.; Levine, M.. Vitamin C as an antioxidant: Evaluation of its role in disease prevention. *Am. J. Clin. Nutr*., **2003**, *22*, 18-35.

[59] Wiegenstam, E.; Rocksen, D.; Ekstrand-Hammarstrom, B.; Bucht, A. Tretment with dexamethasone or liposime-encapsulated vitamin E provides beneficial effect after chemical-induced lung injury. *Inhal. Toxicol*., **2009**, *21*, 958-964.

[60] Tiwari, P.; Kumar, A.; Balakrishnan, S.; Kushwaha, H.S.; Mishra, K.P. Radiation-induced micronucleus formation and DNA damage in human lymphocytes and their prevention by antioxidant thiols. *Mutat. Res*., **2009**, *676*, 62-68.

[61] Kumar, B.S.; Kunwar, A.; Ahmad, A.; Kumbhare, L.B.; Jain, V.K.; Privadarsini, K.I. *In vitro* radioprotection studies of organoselenium compounds: differences between mono- and diselenides. *Radiat. Environ. Biophys.*, **2009**, *48*, 379-384.

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