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Phosgene Oxime: Injury and associated Mechanisms compared to vesicating agents Sulfur Mustard and Lewisite

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Abstract

Phosgene Oxime (CX, Cl_2CNOH), a halogenated oxime, is a potent chemical weapon that causes immediate acute injury and systemic effects. CX, grouped together with vesicating agents, is an urticant or nettle agent with highly volatile, reactive, corrosive, and irritating vapor, and has considerably different chemical properties and toxicity compared to other vesicants. CX is absorbed quickly through clothing with faster cutaneous penetration compared to other vesicating agents causing instantaneous and severe damage. For this reason, it could be produced as a weaponized mixture with other chemical warfare agents to enhance their deleterious effects. The immediate devastating effects of CX and easy synthesis makes it a dangerous chemical with both military and terrorist potentials. Although CX is the most potent vesicating agent, it is one of the least studied chemical warfare agents and the pathophysiology as well as long term effects are largely unknown. CX exposure results in immediate pain and inflammation, and it mainly affects skin, eye and respiratory system. There are no antidotes available against CX-induced injury and the treatment is only supportive. This review summarizes existing knowledge regarding exposure, toxicity and the probable underlying mechanisms of CX compared to other important vesicants' exposure.

Keywords

Phosgene oxime; vesicating agent; nettle agent; skin damage; urticaria; systemic toxicity

1. INTRODUCTION

Chemical warfare agents (CWAs), because of their low cost of manufacturing, easy synthesis and devastating multi-organ toxic effects have been used extensively in warfare (Dacre and Goldman, 1996; Ganesan et al., 2010). The first reported use of CWAs dates

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CONFLICT OF INTEREST

The Authors report no conflicts of interest.

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back to 1915, when chlorine was used by German army against allied forces at Ypres (Ganesan et al., 2010). Subsequently, large quantities of various CWAs (Choking agents, lachrymators, vesicants, nerve agents, and central nervous system-disabling agents) were produced and stockpiled by several nations, which poses an additional accidental exposure risk, apart from their use in conflicts and feared use by terrorists (Geraci, 2008; Saladi et al., 2006; Watson and Griffin, 1992).

Among the various CWAs developed, vesicants/vesicating agents consist of chemicals that lead to the formation of vesicles/blisters apart from their ability to cause acute and debilitating injuries to multiple organs. These include: 1) mustard agents such as sulfur mustard [bis(2-chloroethyl)sulfide; HD; SM; Lost; Yperite; CAS # 505-60-2 (Fig. 1B)], and nitrogen mustards [HN1 (bis(2-chloroethyl) ethylamine; CAS # 538-07-8), HN2 (2,2′-dichloro-*N*-methyldiethylamine; CAS # 51-75-2), and HN3 (tris(2-chloroethyl)amine hydrochloride); CAS # 555-77-1]; 2) arsenical vesicants such as lewisite [L or L-1; LEW; dichloro(2-chlorovinyl) arsine; CAS # 541-25-3 (Fig. 1C)]; and 3) nettle agent phosgene oxime (CX; dichloroformoxime; CAS # 1794-86-1 (Fig. 1A) (TOXNET). Of these, SM has been the most extensively used vesicating agent in various conflicts (Dacre and Goldman, 1996; Saladi et al., 2006), with first reported use in 1917, during the World War I, by the German army against the allied forces near the town of Ypres, Belgium (Prevention, 2011). The year 2017 marks the one hundred years of use of vesicating agent SM in warfare. Extensive use of SM in numerous combats has resulted in large number of causalities which led it to earn the nick name the “King of The Battle Gasses” (Geraci, 2008; McManus and Huebner, 2005).

Despite international efforts to de-arm nations of chemical weapons, multiple countries including Iran, Libya, North Korea, and Syria have huge stockpiles of these agents. Most recently, use of nerve agent, Sarin (GB) against civilians in Syria and the use of SM by Islamic State (ISIL) against Kurdish fighters in Syria, and against civilians in Iraq was reported (Chulov, 2017; Kohnavard, 2016; Nebehay, 2017; SAMSFoundation, 2015). These latest deployments serve as reminder that SM and other CWAs still pose a potential threat and could be used by individuals/groups motivated to cause mass casualties, highlighting the need to step up research efforts to understand the injury mechanisms and to develop targeted therapies.

SM has been the most extensively studied vesicating agent (Dacre and Goldman, 1996; Ghabili et al., 2011; Ghabili et al., 2010). A variety of animal models including mice, rabbits, rats, weanling pigs, and hairless guinea pigs, have been used to study the toxicity and pathology of SM, and to understand the underlying mechanisms (Allon et al., 2009; Banin et al., 2003; Dachir et al., 2010; Greenberg et al., 2006; Joseph et al., 2011; Kadar et al., 2009; Kadar et al., 2001; Kan et al., 2003; Morad et al., 2005; Paromov et al., 2007; Petrali et al., 2000; Shakarjian et al., 2010; Smith et al., 1997; Smith et al., 1995). Monofunctional analogs such as 2-chloroethyl ethyl sulfide (CEES) and bifunctional analogues, NMs, have been used to study SM induced toxicity and associated mechanisms as synthesis and use of SM is highly restricted (Banin et al., 2003; Black et al., 2010; Gordon et al., 2010; Goswami et al., 2015; Goswami et al., 2016b; Han et al., 2004; Hardej and Billack, 2007; Inturi et al., 2011; Jain et al., 2011a; Jain et al., 2014a; Jain et al., 2011b;

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Jowsey et al., 2009; Mangerich et al., 2016; O'Neill et al., 2011; Rancourt et al., 2012; Tewari-Singh et al., 2011; Tewari-Singh et al., 2010; Tewari-Singh et al., 2014; Tewari-Singh et al., 2012).

Arsenical vesicating agent LEW was developed as a chemical warfare agent during World War I. Though not used in warfare, its stockpiles are known to exist and it has been reported to be mixed with SM or other chemical warfare agents to achieve greater effectiveness in combat (Goldman and Dacre, 1989; Kohnavard, 2016; Tewari-Singh et al., 2016). Compared to SM, there are fewer studies on the toxicity mechanisms of LEW (Li et al., 2016b; Mann et al., 1946, 1947; Mouret et al., 2013; Nguon et al., 2014; Tewari-Singh et al., 2016; TOXNET).

The nettle agent and vesicant CX is reported to be stockpiled during World War II as a potent chemical weapon which could be used alone or with other chemical warfare agents to cause startlingly rapid incapacitation and death. Though, this is the most dangerous chemical agent among the vesicants, it is the least studied agent (Augerson, 2000; Patocka, 2011; Tewari-Singh et al., 2017) (Table 1).

Exposure to vesicating agents causes damage to the ocular, skin and pulmonary systems at even at low doses while higher dose exposures lead to multi-organ toxicity including systemic effects (Augerson, 2000) (Table 2). Mustard agents cause acute and chronic debilitating injuries from ocular and dermal absorption, as well as lung inhalation, resulting in severe injury to these tissues as well as systemic toxic effects including the gastrointestinal, hematological, immunological, mucoskeletal, reproductive, nervous, and cardiac systems at higher exposure doses (Ghabili et al., 2011; Ghasemi et al., 2013; Graham and Schoneboom, 2013; Panahi et al., 2013). Injury from SM is biphasic with symptoms of delayed injury appearing as long as 40 years after the initial exposure (Balali-Mood and Hefazi, 2006; Balali-Mood et al., 2008; Etezad-Razavi et al., 2006; Ghabili et al., 2010; Ghanei et al., 2010; Hefazi et al., 2006; Kehe and Szinicz, 2005; Keramati et al., 2013; Korkmaz et al., 2008; Shohrati et al., 2007) (Table 2). At the molecular level, these effects could be attributed to SMs alkylating properties and/or thiol-depleting properties, resulting in the activation of signaling pathways related to DNA damage, oxidative stress, and inflammation (Kehe et al., 2009; Paromov et al., 2007; Sabourin et al., 2002; Shakarjian et al., 2010) (Table 3).

Arsenical vesicant LEW exposure also causes debilitating effects on its primary target organs eyes, skin and the respiratory systems with more severe lesions. However, as compared to mustard vesicants, its toxicity is associated with severe pain within minutes of exposure and its faster cutaneous absorption causes more sever systemic effects (Li et al., 2016a; Mouret et al., 2013; Nguon et al., 2014; Tewari-Singh et al., 2016; TOXNET)(Table 2). However, there is limited information available on the toxic effects of LEW (Augerson, 2000; Goldman and Dacre, 1989; McManus and Huebner, 2005; Prevention, 2011). The toxic outcomes of LEW exposure could be attributed to its ability to combine with thiol groups, react with biological sulfhydryl groups and glutathione, and to release hydrochloric acid. At molecular level, oxidative stress, unfolded protein response, inflammation and apoptosis in addition to heavy metal toxicity are plausible mechanisms responsible for LEW

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toxicity (CDC, 2011; Li et al., 2016b; Mouret et al., 2013; Nguon et al., 2014; Tewari-Singh et al., 2016) (Table 3).

CX was first synthesized in 1929 and its potential as a chemical warfare agent was recognized due to its fast penetration and immediate injuries (Patocka, 2011). Although it was stockpiled during World War II, there are no records of its use in battlefield. It was produced alone or as a mixture with LEW and SM to enhance their penetration. CX is a halogenated oxime and is a colorless, crystalline solid with a strong, disagreeable odor and violently irritating vapor (Patocka, 2011; Schraga, 2016). CX is generally produced by reduction of chloropicrin by tin in presence of hydrochloric acid (Patocka, 2011). CX exists in vapor form at ambient atmosphere as it has a vapor pressure of 11.2 mmHg at 25 °C. It is heavier than air thus settles in low-lying areas. CX is relatively non-persistent in soil, as it is highly unstable and decomposes rapidly before it could volatilize. CX is expected to have high mobility in soil due to a soil adsorption coefficient (Koc, based on structure estimation method) of 68, and it exists partially as an anion as it's a weak acid (estimated pKa 6.5) (Meylan et al., 1992; Swann et al., 1983). It is soluble in water and organic solvents and hydrolyses very rapidly, particularly in the presence of an alkali to form hydrogen chloride and hydrolamines (Bartelt-Hunt et al., 2006b; Ellison, 2007; Wismer, 2009). CX has a reported half-life of 83 days at unspecified pH and temperature (Bartelt-Hunt et al., 2006a). Volatilization of CX from aqueous solutions is not expected to be a major fate because of its presence as an anion. Bioconcentration is not expected as CX has an estimated bioconcentration factor (BCF) of 3. (ATSDR, 2014; Augerson, 2000; CDC, 2011). Physical and chemical properties of CX, SM and LEW are summarized in Table 1.

Although CX is grouped together with vesicants, this is an urticant or nettle agent and not a pure vesicant as it does not lead to blister/vesicles formation. It produces intense itching and rash resembling hives upon cutaneous exposure (Augerson, 2000; Patocka, 2011). Its exposure in both liquid and vapor forms can cause more severe damage to the skin, eye, and lung tissues than other vesicants due to its fast penetration, immediate pain and tissue destruction. In addition, its rapid absorption through the skin can lead to immediate skin damage and severe systemic toxicity that can lead to rapid mortality. The nature of injuries caused by CX resembles those caused by acids, therefore it is often referred to as a corrosive agent (Patocka, 2011). The mechanism of action of CX is unknown; however, it likely possesses alkylating and nucleophilic properties resembling mustard vesicants, and its effect could be direct involving corrosive injury, cell death and tissue destruction, or indirect involving inflammatory response causing delayed tissue injury. Unlike SM and LEW, reports on the effects of CX exposure and the mechanism of injury, and long term effects are unknown (Augerson, 2000). We have employed SKH-1 hairless mouse model in our recently published report to understand CX-induced acute injury and the underlying molecular mechanisms (Tewari-Singh et al., 2017). Further studies are needed to understand the injury mechanism of the rapid onset of severe and prolonged effect of CX exposure to develop effective therapies against this most potent vesicant.

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2. INJURY SYMPTOMS & TARGET ORGANS

The injury symptoms upon vesicants' exposure vary depending on the dose, route and form of exposure. CX is absorbed rapidly and has faster penetration (it can even penetrate rubber gloves) than other vesicating agents. The symptoms upon CX exposure appear instantly in comparison to SM or LEW; it takes few seconds to minutes for the symptoms to appear upon LEW exposure, and for SM, the latency period is in hours (Augerson, 2000). A comparison of injury symptoms upon CX, SM, and LEW exposure is summarized in Table 2. Skin and mucous membrane irritation can begin within seconds of exposure to low doses of CX (0.2mg.min/m^3), while unbearable pain and irritation could occur minutes after exposure to a dose of 3mg.min/m^3. Lethal systemic dose estimation [LCt50 (concentration-time product capable of killing 50% of exposures)] is 1500–2000mg.min/m^3 (ATSDR, 2014; Schraga, 2016).

The rapid skin damage caused by CX makes the skin susceptible to injury from other chemical agents. CX exposure at higher concentrations is more damaging and causes instant pain followed by tissue necrosis, systemic effects and mortality (Augerson, 2000). People could be exposed to CX by air, by breathing the gas and skin and eye contact. If liquid CX is released into the water or food, people can be exposed by drinking water or eating the contaminated food (Patocka, 2011). Immediate eye and respiratory irritation occurs upon CX vapor exposure. The symptoms include cough, throat pain, increased lachrymation and impaired vision (Augerson, 2000).

3.1. Eye

Eyes are the most sensitive organ to vesicant exposure (Ghasemi et al., 2013; Gordon et al., 2009; Goswami et al., 2016a; Kadar et al., 2013a; Kadar et al., 2009; Kadar et al., 2013b; McNutt et al., 2012; Tewari-Singh et al., 2016). CX exposure of the eye results in immediate and severe pain, irritation, edema, lacrimation, conjunctivitis, and blepharospasm with more severe exposure resulting in keratitis, iritis, corneal perforation and blindness (Table 2) (Patocka, 2011). Unlike SM, there are no reports available on the long-term ocular effects of LEW and CX. SM exposure is known to cause a biphasic injury with the symptoms appearing few hours after the exposure and comprising of an acute phase with inflammation, conjunctivitis, lachrymation, photophobia, keratitis, corneal ulceration and erosion and a delayed phase consisting of persistent epithelial defects, keratitis, corneal scarring, neovascularization, endothelial cell damage and limbal stem cell deficiency (Gordon et al., 2009; McNutt et al., 2012). LEW exposure of the eyes is reported to cause instant pain, inflammation, irritation, swelling and tearing, edema of eyelids, massive corneal necrosis and blindness (Goldman and Dacre, 1989; Olajos et al., 1998; Tewari-Singh et al., 2016).

3.2. Respiratory system

CX is quickly absorbed upon inhalation exposure causing immediate and incapacitating irritation, pain, runny nose, hoarseness, as well as local tissue destruction of the upper airways at low exposure doses, while serious complications such as pulmonary edema followed by tachypnea, dyspnea, and cyanosis occur at higher exposure doses (Augerson, 2000; Patocka, 2011; Schraga, 2016). Exposure to aerosol could result in necrotizing

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bronchiolitis, pulmonary edema with pulmonary vein thrombosis (Augerson, 2000). Unlike SM, the long term respiratory effects of CX are unknown, although it is believed to result in the development of pulmonary fibrosis (Augerson, 2000). In comparison, the respiratory injury symptoms from SM exposure include sneezing, nasal and throat irritation, loss of taste and smell at lower exposures doses, while higher dose exposure results in laryngitis, aphonia, bronchitis, coughing, pseudomembrane formation, dyspnea, and hypoxia (Weinberger et al., 2016; White et al., 2016). Long term effects of SM exposure include chronic bronchitis, decreased lung capacity, pulmonary fibrosis, and increased incidences of lung cancer (Balali-Mood et al., 2008; Beheshti et al., 2006; Ghanei et al., 2008; Ghanei and Harandi, 2007; Ghasemi et al., 2013; Hefazi et al., 2005). Respiratory symptoms upon LEW exposure resemble those of upper respiratory infections, with sneezing, nausea, coughing, rhinitis, mucous membrane erythema. Severe LEW exposure results in coughing, laryngitis and aphonia (Augerson, 2000; McManus and Huebner, 2005; TOXNET).

3.3 Skin

CX is absorbed quickly upon dermal exposure and results in immediate itching, pain, skin blanching, erythema, edema, hives formation, pruritic, pigmentation and severe necrosis. Desquamation with necrosis of the skin could be followed by eschar formation and polymorphonuclear infiltrates and the complete healing could take months (Augerson, 2000; Tewari-Singh et al., 2017). Severe exposure of the skin could result in systemic effects, but long-term effects are unknown (Augerson, 2000; Patocka, 2011; Tewari-Singh et al., 2017). Exposure of the skin to vesicant SM results in acute and chronic lesions, with varying severity and symptoms depending on the dose and duration of the exposure. SM is a very lipophilic molecule and readily penetrates the skin. Appearance of symptoms could take few hours to days and include edema, erythema, inflammation, epidermal-dermal separation, blistering, ulceration, desquamation, and necrosis. Delayed effects include altered pigmentation, presence of cherry angiomas, eczema, hypertrophy, and dry and sensitive skin (Balali-Mood and Hefazi, 2005, 2006; Dacre and Goldman, 1996; Ghabili et al., 2011; Ghabili et al., 2010). LEW exposure of the skin results in immediate itching, erythema, edema, desquamation, inflammation, vesication, and degenerative necrotic changes (Augerson, 2000; Li et al., 2016b; Mouret et al., 2013; Nguon et al., 2014).

3.4. Systemic toxicity

Apart from the toxic effects of CX exposure on eyes, respiratory, and skin system, CX exposure has also been shown to induce severe systemic toxic effects in our recent study (Tewari-Singh et al., 2017). Histopathological analyses of the lung, liver, spleen, kidney, and heart tissue showed dilatation of the peripheral vessels (including capillaries and sinusoids) and the pooling of red blood cells (RBCs) in the vessels (Tewari-Singh et al., 2017). This severe vascular dilation could result in a marked loss of blood from the vessels into the surrounding tissue and could lead to low blood pressure, relative hypoxia, and shock leading to possible mortality. Similar effect has been observed for high dose exposure of LEW, where death may result from fluid loss, hypovolemia secondary to capillary leakage - known as the “Lewisite shock” (Smith et al., 1997; Watson and Griffin, 1992). Higher dose exposures of SM affect the rapidly-dividing cells in the GI tract and bone marrow. In the GI tract, destruction of the mucosa and perforations of the GI walls lead to GI bleeding

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(Ghasemi et al., 2013), while in the bone marrow, it results in bone-marrow suppression. Pancytopenia, increase in the number of RBCs and hematocrit in long-term, loss of spleen cells, depression of cell-mediated immunity are also observed. SM is also classified as a carcinogen and few reports have shown that it affects the reproductive, cardiovascular, renal, hepatic, and central nervous system and causes psychological complications (Balali-Mood and Hefazi, 2005; Geraci, 2008; Ghabili et al., 2010; Ghasemi et al., 2013).

3. PATHOPHYSIOLOGY

Among vesicants, molecular mechanism of SM toxicity has been extensively studied. SM is a highly reactive chemical and in aqueous solutions forms the sulfonium ion that reacts readily with all major macromolecules in the cell (Dacre and Goldman, 1996; Kehe et al., 2009). DNA damage is one of the key events after SM exposure leading to H2A.X and p53 phosphorylation (Joseph et al., 2011; Paromov et al., 2007). PARP activation leads to cellular NAD^+ depletion, cell cycle arrest, and activation of DNA damage repair. The cell could undergo apoptosis/necrosis depending upon the extent of damage. SM is also known to cause ER stress resulting in changes in Ca^{++} homeostasis, reduction in cellular glutathione levels, NO signaling and oxidative stress. Release of inflammatory mediators like cyclooxygenase-2 (COX-2) and cytokines [tumor necrosis factor- alpha (TNF-α), interleukins (IL)-1α/β, IL-6 and IL-8) as well as activation of matrix metalloprotease-9 (MMP-9), Nuclear factor-kappa B (NF-κB) and mitogen activated protein kinase (MAPK) pathways are also reported to play a major role in SM-induced toxicity (Dacre and Goldman, 1996; Kehe et al., 2009; Mouret et al., 2015; Shakarjian et al., 2006; Shakarjian et al., 2010) (Table 3). Similarly, in LEW injury, DNA damage, apoptotic cell death, unfolded protein response (UPR) pathway, oxidative stress, decrease in cellular glutathione levels, release of inflammatory mediators (COX-2) and cytokines (TNF- α, IL-1β, IL-6, and IL-8), and activation of MMP-9 and NF-κB pathways have been reported. Arsenic poisoning and inhibition of carbohydrate metabolism are also reported to be involved in LEW- induced toxicity (Augerson, 2000; Goldman and Dacre, 1989; Kehe et al., 2001; Li et al., 2016b; Mouret et al., 2013; Nelson et al., 2006; Nguon et al., 2014; Srivastava et al., 2016) (Table 3). There are very few reports on the toxic effects of CX, and the mechanism of injury is unknown. Possible molecular mechanisms involved in SM-, LEW- and CX-induced injury have been summarized in Table 3. The toxic effects upon CX exposure could be attributed to its alkylating properties, or to the effect of chlorine, oxime or carbonyl groups, resulting in direct (enzyme inactivation, corrosive injury and cell death with rapid tissue destruction) or indirect toxicity (involving activation of alveolar macrophages, recruitment of neutrophils, and release of hydrogen peroxide), resulting in delayed tissue injury, such as pulmonary edema (Augerson, 2000; Tewari-Singh et al., 2017).

In our recent report, we analyzed the effect of acute cutaneous phosgene-exposure in SKH-1 hairless mice. Cutaneous CX exposure (4 min exposure on two 12-mm sites on the dorsal surface) resulted in 20% mortality within 8 h. In the exposed skin area, blanching was observed within minutes of exposure with the center surrounded by an erythematosus ring. Urticaria (red hives-like area), necrosis and wheal formation was also observed within the blanched skin area (Tewari-Singh et al., 2017). Immediate increase in skin injury parameters, including doubling of skin bi-fold thickness, moderate erythema and edema, and

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necrosis was observed that maxed at 2h post-exposure. Histopathologic analyses were consistent with the observed injury parameters and were similar to skin urticaria due to allergic and non-allergic reactions to various environmental substances (Jain, 2014). In addition, an increase in the inflammatory cells mostly neutrophils and degranulated mast cells was observed. The neutrophil infiltration was further confirmed by increased myeloperoxidase (MPO) expression in the skin lysates. Neutrophil infiltration has also been shown to play a key role in skin inflammation related to mustard vesicating agents (Jain et al., 2014b; Shakarjian et al., 2010; Wormser et al., 2005).

DNA damage, p53 phosphorylation and accumulation have also been shown to play an important role in vesicating agents-induced apoptotic cell death, and have also been associated with the injury (Goswami et al., 2016b; Kehe and Szinicz, 2005; Paromov et al., 2007). Like mustards, an increase in phosphorylation of p53 at ser15 and its accumulation in the skin tissue samples upon CX exposure were observed. Cutaneous exposure to CX also resulted in an increase in TNFα and COX-2 levels in the skin tissue, which has been also observed with vesicating agents exposure of the skin tissue (Shakarjian et al., 2010; Tewari-Singh et al., 2017; Tewari-Singh et al., 2009).

The systemic effects seen in our reported study with CX further support the fact that CX is absorbed instantaneously, leading to more severe systemic toxicity and death compared to other vesicating agents. Although, cutaneous exposure to other vesicants at higher doses, results in damage to multiple organ systems but mortality is rare (Dacre and Goldman, 1996; Goswami et al., 2015; Kehe et al., 2008; Patocka, 2011).

4. TREATMENT

Among vesicating agents, effective anti-dotes are available only for LEW-induced toxicity, in the form of British Anti-Lewisite (BAL; dimercaprol) and derivatives, meso-2,3-dimercaptosuccinic acid (DMSA) and 2,3-dimercapto-1-propane- sulphonic acid (DMPS) (Goswami et al., 2016a; Hughes, 1946, 1947; Mann et al., 1947; Mouret et al., 2013). However, there are still limitations with the use of these therapies including narrow therapeutic window, toxicity and difficulty in administration, thus, requiring the need for the development of better and safe antidotes. There are no effective approved antidotes available for SM. Although a number of compounds including anti-oxidants, protease inhibitors, PARP inhibitors, angiogenesis inhibitors, calcium modulators, anti-inflammatory agents, and flavanones have been shown to be effective to various extents in laboratory studies (Balszuweit et al., 2013; Goswami et al., 2016a; Kadar et al., 2014; Kadar et al., 2009; Laskin et al., 2010; McElroy and Day, 2016; Paromov et al., 2007; Smith, 2009; Tewari-Singh and Agarwal, 2016; Weinberger et al., 2016). There is no specific antidote available against CX-induced injuries and the treatment is mostly supportive to reduce symptoms, prevent infections and help healing. For oral exposures, dilution with water or milk could be helpful. For ocular injury, irrigation with copious amount of water could be helpful while for necrotic skin lesions, surgical intervention may be required. Recovery depends on the extent of injury and could take anywhere several months (Patocka, 2011). Since CX is absorbed within seconds, the timing of decontamination is very crucial. Systemic analgesics are preferred over topical anesthetics, as use of later may increase the severity of corneal

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damage (Schraga, 2016). Our recently published study shows that some of the molecular events upon CX-induced injury, could be similar to those induced by mustard-induced toxicity, including p53 phosphorylation and accumulation, increased COX-2 and TNFα levels, and increased MPO activity (Joseph et al., 2011; Mouret et al., 2015; Smith et al., 1997; Tewari-Singh et al., 2017; Tewari-Singh et al., 2013). Hence, agents identified for treating mustard induced injuries could also be tested as potential therapies for CX-induced injury. Since mast cell activation and histamine release could be involved in CX-induced instantaneous inflammation and urticarial; anti-histamine, anti-inflammatory and immunosuppressant drugs could useful to reduce the inflammatory response and mortality associated with CX-induced toxicity (Hennino et al., 2006; Jain, 2014; Tewari-Singh et al., 2017). Analgesics and antibiotics could be given to reduce the pain and prevent infections and promote healing.

5. CONCLUSIONS

CX is a dangerous, corrosive, and fast penetrating urticant, which can cause serious immediate toxic effects and incapacitation with fast mortality due to systemic effects. It is known to cause more severe tissue damage than other vesicating agents; however, its toxicity has not been well studies and its mechanism of action is unknown. Although it has never been used in warfare, its potent nature and toxic consequences make it a potential military and terrorist weapon. It could be produced as a weaponized mixture with other chemical warfare agents to enhance their deleterious effects. There are no antidotes available for CX, only removal of causalities from the source of exposure and rapid decontamination are the key factors in reducing causalities. Further comprehensive studies to investigate pathophysiology of the toxic effects of CX are needed to develop effective therapies.

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ABBREVIATIONS

BAL	British Anti-Lewisite (dimercaprol)
COX-2	cyclooxygenase-2
CWAs	Chemical warfare agents
CX	phosgene oxime (dichloroformoxime)
DMPS	2,3-dimercapto-1-propane- sulphonic acid
IL	Interleukins
LEW	Lewisite; dichloro(2-chlorovinyl) arsine
MAPK	mitogen activated protein kinase

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MMP-9	matrix metalloprotease-9
MPO	myeloperoxidase
NM	nitrogen mustard
RBCs	red blood cells
SM	sulfur mustard
TNF-α	tumor necrosis factor- alpha
UPR	unfolded protein response

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TOXNET, Phosgene Oxime.

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Highlights

- Phosgene oxime (CX) is a nettle agent grouped together with vesicants.
- CX is the most potent but least studied vesicating agent.
- CX is absorbed quickly and causes immediate pain and inflammation.
- CX with faster penetration causes severe tissue damage and systemic toxicity.
- CX could be produced as a weaponized mixture with other chemical warfare agents.

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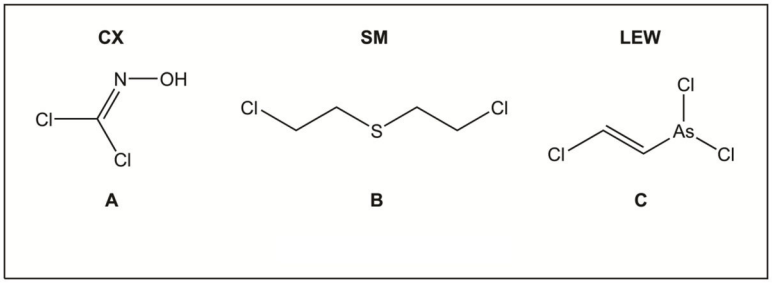

Figure 1.

Chemical structures of nettle vesicant CX (A), mustard vesicant SM (B), and arsenical vesicant LEW (C). The structures were drawn using Chem Draw software (Chem Draw Professional 17.0).

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Table 1

Physical and chemical properties of nettle vesicant CX (A), mustard vesicant SM (B), and arsenical vesicant LEW (C).

Agent	Phosgene oxime CX Dichloroformoxime	Sulfur mustard Bis(2-chloroethyl) sulfide, SM, HD, Yperite, LOST	Lewisite Agent L Dichloro (2-chlorovinyl) arsine
Molecular weight	113.93	159.07	207.31
Physical state (at 20 °C)	colorless, crystalline solid or yellowish-brown liquid (munitions-grade)	Oily, colorless (pure) to yellowish dark-brown (munitions-grade) liquid	Oily colorless liquid
Aqueous solubility	Soluble	Slightly soluble	Slightly soluble
Vapor density (compared to air)	3.9	5.4	7.1
Vapor pressure (mm Hg at 20 °C)	11.2	0.06–0.11	0.395
Volatility (mg/m^3 at 20 °C)	1800	610	4480
Boiling point (°C)	128	215–217	190
Melting point (°C)	35–40	13–14.4	0.1
Decomposition temperature (°C)	<128	149–177	>100
Odor	Disagreeable, prickling odor	Almost odorless (in pure state at typical field concentrations); horseradish, garlic or mustard odor at higher concentrations	Faint geranium like odor

Sources: (Augerson, 2000; Dacre and Goldman, 1996; TOXNET)

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Table 2

Injury symptoms upon exposure to nettle vesicant CX (A), mustard vesicant SM (B), and arsenical vesicant LEW (C).

Ocular injury	Immediate pain, conjunctivitis, edema, keratitis, iritis, lacrimation, vision loss, temporary blindness	**Acute/early**: Irritation, foreign body sensation, pain, conjunctivitis, photophobia, edema, corneal ulceration, opacity, lacrimation, blepharospasm, vesication, temporary blindness **Chronic/delayed**: Vasculitis, corneal scarring, opacification, ulceration, perforation and erosions, limbal stem cell deficiency, neovascularization, endothelial cell damage	Immediate pain, blepharospasm, conjunctivitis, photophobia, lacrimation, corneal ulceration and opacity, iritis, temporary blindness
Pulmonary injury	Irritation, pulmonary edema, necrotizing bronchitis, pulmonary venule thrombosis	**Acute/early**: Coughing, choking, dyspnea, hypoxia, pseudomembrane formation, bronchospasm, pulmonary edema, rhinorrhea, tachypnea **Chronic/delayed**: Decreased lung capacity, pulmonary fibrosis, increased incidences of lung cancer, emphysema, chronic bronchitis, bronchiolitis	Sneezing, coughing, rhinitis, pulmonary edema
Cutaneous injury	Immediate itching, erythema, edema, hives, blanching (skin whitening), urticaria, tissue necrosis/ eschar	**Acute/early**: Itching, erythema, blisters/vesication, desquamation, hyperesthesia, necrosis/eschar, pruritis, purpura, hyper/hypo-pigmentation **Chronic/delayed**: Atrophy, scarring, eczema, popular rash, keloids, cherry angiomas, scaling, seborrheic dermatitis	Immediate itching/ stinging followed by erythema, blisters/ vesication
Other organ systems affected	GI tract, liver, cardiovascular, kidneys, spleen, and Immune system	GI tract, liver, cardiovascular, CNS, kidneys, spleen, immune system, bone marrow, lymphatic, reproductive, and mucoskeletal system	GI tract, liver, cardiovascular, and CNS

Sources: (Augerson, 2000; Ghabili et al., 2010; Ghasemi et al., 2013; Goldman and Dacre, 1989; Graham and Schoneboom, 2013; Kadar et al., 2009; Mouret et al., 2013; Nguon et al., 2014)

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Table 3

Molecular mechanisms involved in nettle vesicant CX (A), mustard vesicant SM (B), and arsenical vesicant LEW (C) induced toxicity.

CX	SM	LEW
• p53 phosphorylation (Ser15) and accumulation, DNA damage • Apoptosis • Inflammation (Mast cell degranulation, increased MPO activity, increased COX-2, and TNF-α expression) • Necrosis	• H2A.X phosphorylation (Ser139), p53 phosphorylation (Ser15) and accumulation, DNA damage • Apoptosis • Inflammation [MAP kinases, NF-κB activation, increased COX-2 expression, and cytokine release (IL-1α/β, IL-6, IL-8, and TNF-α)] • Oxidative and nitrosative stress • Proteolytic activation (Matrix-metalloproteases or MMPs and serine proteases) • Ca imbalance (ER stress) • PARP activation • Necrosis	• Apoptosis • Inflammation (NF-κB activation, increased COX-2 expression, and cytokine release (IL-1β, IL-6, IL-8, and IFN-α) • Oxidative stress • Proteolytic activation (Matrix- metalloproteases or MMPs) • Activation of UPR signaling pathway

Sources: (Ghabili et al., 2011; Gordon et al., 2010; Kehe et al., 2009; Kohnavard, 2016; Laskin et al., 2010; Li et al., 2016b; Nguon et al., 2014; Paromov et al., 2007; Shakarjian et al., 2010; Srivastava et al., 2013; Srivastava et al., 2016; Tewari-Singh et al., 2017).