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Ocular toxicity of mustard gas: a concise review

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Abstract

Sulfur mustard (SM) is a chemical warfare agent that has been used throughout recent history and remains a threat today. Exposed soldiers and civilians experience a variety of symptoms primarily in the respiratory system, skin, and eyes. The ocular tissues are highly sensitive to damage by SM and undergo unique manifestations of acute, chronic, and delayed complications that can persist for months and years after exposure. The mechanisms of this unique mustard gas keratopathy are still not fully understood and animal models for the study of this disease are discussed. Recent advances in mechanisms of injury are included in this review. Ophthalmic manifestations of SM injury including persistent epithelial defects, limbal stem cell deficiency, corneal neovascularization, dry eye, and corneal opacification have been reported. A wide variety of medical and surgical therapies have been studied and are reviewed here along with potential future therapies.

Keywords

Sulfur mustard; Mustard gas keratopathy; cornea; corneal stromal injury

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Declaration of interest

None of the authors has any competing interests.

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INTRODUCTION AND OVERVIEW

Sulfur mustard (SM) gas is a potent vesicating agent that has been used multiple times in the past 100 years as a devastating chemical warfare agent. The earliest reports of mustard gas usage date back to World War 1 and the most recent was 2016 in Syria (Baradaran-Rafii et al., 2011; Blodi, 1971; Javadi et al., 2005; Panahi et al., 2017b; Safarinejad et al., 2001). Presently, known stockpiles of SM exist worldwide (Wolfe et al., 2019). Sulfur mustard is classified as a schedule 1 agent which can affect large numbers of people simultaneously. Ocular injuries were reported in 90% or more of exposed victims and symptoms may be observed months to years after exposure in some cases (Ghasemi et al., 2009; Javadi et al., 2007; Safarinejad et al., 2001; Solberg et al., 1997).

Mustard gas is a pale yellow, oily, highly toxic, volatile, liquid alkylating chemical with a mustard or garlic odor. It evaporates rapidly to a poisonous gas and exists as an aerosolized liquid. Sulfur mustard is a thick liquid at ambient temperature, but becomes a solid at 58 °F. Due to its highly reactive, lipophilic nature, SM rapidly penetrates and is absorbed by many tissues but damage to the eyes, skin, respiratory tract, and gastrointestinal and immune systems are particularly rapid and severe (Balali-Mood and Hefazi, 2005). In the eyes, the cornea and other ocular surface tissues are highly susceptible to damage by SM contact and people experience painful and short- and long-term blinding conditions (Dahl et al., 1985; Mann and Pullinger, 1942; Solberg et al., 1997). Immediate, acute injury occurs even with low levels of exposure. At higher levels of exposure, patients can develop chronic corneal pathology, which can occur shortly after initial apparent resolution or months to years later (Shoeibi et al., 2017; Shohrati et al., 2007; Solberg et al., 1997). Together, these signs comprise the clinical condition known as mustard gas keratopathy (MGK), with biphasic acute and delayed-onset manifestation of symptoms via a variety of mechanisms. A variety of medical and surgical treatments have been published to manage ocular injuries from mustard gas exposure, but currently there is no effective curative therapy (Balali-Mood and Hefazi, 2005; Panahi et al., 2017b; Rajavi et al., 2017; Razavi et al., 2012; Safarinejad et al., 2001; Solberg et al., 1997; Wolfe et al., 2019).

OCULAR MANIFESTATIONS OF MGK

The acute ocular symptoms of SM injury vary depending on the duration and level of exposure. Signs may appear within 30 minutes of exposure and subside within 2–6 weeks depending on severity. General acute symptoms may include eye pain (e.g. blepharospasm), burning, conjunctivitis, photophobia, lacrimation, and decreased vision (Balali-Mood and Hefazi, 2005, 2006; Baradaran-Rafii et al., 2011; Blodi, 1971; Kehe et al., 2009; Solberg et al., 1997). Classification of acute injury is subdivided based on the overall concentration of exposure and was first described based on battlefield exposure doses (Solberg et al., 1997). Mild injury occurs with doses of $<80 mg/min/m^3$ and results in conjunctival injection and ocular irritation between 4–24 hours after exposure. The cornea is generally spared and there is resolution of signs within a few days to 1–2 weeks. Moderate injury occurs at exposure to larger doses of 100–200 $mg/min/m^3$ characterized by the symptoms of mild exposure, accompanied by severe ocular pain, corneal edema, corneal bullae formation, and temporary blindness within 3–6 hours after exposure. Photophobia, chemosis, and

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blepharitis are additional signs that may accompany moderate exposure levels. Resolution may take 2–6 weeks. Severe ocular symptoms are present at exposure to SM doses of >200mg/min/m^3 and include the initial symptoms of mild to moderate exposure, followed by progression to include uveitis, conjunctival and limbal necrosis, corneal ulcerations, and sometimes permanent blindness. Onset of clinical signs is rapid, and though healing may appear to begin after several weeks, these cases are more likely to develop late complications and chronic or recurrent signs that last for months or years (Borak and Sidell, 1992; Javadi et al., 2005; Panahi et al., 2017a; Rowell et al., 2009; Solberg et al., 1997).

After the acute phase, three clinical courses are possible: resolution, chronic MGK, and latent MGK. The majority of patients experiencing mild or moderate exposures to SM will undergo complete resolution. However, a subset of patients, particularly those with high exposure levels, will experience either chronic or latent (delayed-onset) forms (Balali-Mood and Hefazi, 2005; Blodi, 1971; Borak and Sidell, 1992; Javadi et al., 2005; Solberg et al., 1997). The collective pathology of chronic/delayed onset symptoms is known as mustard gas keratopathy and the etiology is still not fully understood.

Chronic MGK represents a continuum from acute injury without complete resolution of signs. Latent injury represents apparent resolution, however signs subsequently re-emerge after an asymptomatic period ranging from several weeks to as much as 40 years in one case report (Javadi et al., 2005; Solberg et al., 1997). MGK has been diagnosed in as many as 16% of patients with moderate or severe MG exposures (Khateri et al., 2003; McNutt et al., 2012).

CURRENT INVESTIGATIVE ANIMAL MODELS

A variety of models have been developed to study SM injury in a laboratory setting. Nitrogen mustard is a useful, readily available bi-functional SM-analog and has been widely used and reported to produce similar injuries, though less severe than those of SM (Banin et al., 2003; Gonzalez et al., 1995; Goswami et al., 2019; Goswami et al., 2018; Tewari-Singh, 2014; Tewari-Singh et al., 2012). However, SM itself is still often used in the research setting with appropriate precautions.

Mice have been utilized in some studies, due to historical availability of reagents for many of the corneal markers selected for study in SM-injury (Amitai et al., 2005; Meng et al., 2019; Ruff et al., 2013). Rabbits have also been a widely used model system for SM injury in people. Rabbits have anatomical and physiological features that make them ideal for ocular toxicology research, including large cornea to sclera ratio, and a greater similarity to human eyes than other laboratory animals such as mice or rats (Milhorn et al., 2010). Although rabbits are more resistant to SM injury than humans, previous studies have established normalized doses and a similar progression of injury after SM exposure as observed in exposed humans (Kadar et al., 2001; Mann and Pullinger, 1942; McNutt et al., 2012; Milhorn et al., 2010). It is important to note that they are not a perfect animal model correlate, as rabbits are able to regenerate corneal endothelial cells to an extent, while humans have no regenerative endothelial cell capabilities (Joyce et al., 1996).

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Initial studies utilized liquid SM applied to the rabbit cornea in droplet form (Mann and Pullinger, 1942; Petrali et al., 2000). This resulted in a localized ulcerative injury dissimilar to the typical vapor injuries seen in combat veterans. These studies helped establish that a larger relative dose was needed in rabbit eyes compared to the doses observed to cause lesions in humans. In addition, a condensed time to development of lesions in rabbits was noted. This accelerated lesion development is an additional benefit to using rabbits as a study model.

Based on these studies, there is a reliable pattern of ocular clinical manifestations of MGK in rabbits after exposure to SM which is classified into three temporal phases: 1) there is an acute phase lasting 1–2 weeks after injury; 2) a latent phase in which clinical signs re-appear several weeks after apparent resolution; and 3) a delayed phase in which signs re-appear 3–5 weeks after initial exposure and resolution (Goswami et al., 2019; Kadar et al., 2001; McNutt et al., 2012; McNutt et al., 2013; Milhorn et al., 2010; Petrali et al., 2000; Tewari-Singh, 2014; Tewari-Singh et al., 2012). This well-established pattern is similar to the MGK phenomenon clinically observed in humans after SM exposure, but in a condensed time format.

Doses of SM used in rabbits that will produce mild and moderate lesions have been reported as 370ug/L to 420ug/L, with the higher doses more likely to cause delayed phase lesions (Kadar et al., 2001; McNutt et al., 2012). Several vapor models have been developed to more closely resemble battlefield injuries, and generally exposure times of 1 to 3 min are used as this causes an acute injury that seems to resolve within 1–2 weeks, followed by a high frequency of recurrent (delayed) injury several weeks later (Etezad-Razavi et al., 2006; Kadar et al., 2001; Lee and Chung, 2012; McNutt et al., 2012; McNutt et al., 2013; Milhorn et al., 2010; Tewari-Singh et al., 2012).

One such goggle-based delivery system was developed to more closely resemble battlefield injuries, ensuring SM injury to the entire cornea and ocular adnexa (Kadar et al., 2001). This technique developed by Kadar *et. al.* used distilled liquid SM to produce a known concentration of sulfur mustard vapor inside of a glass goggle using concentrated air flow. Their goal was to mimic battlefield conditions, and as such, exposed both eyes (Kadar et al., 2001). More recent studies focusing on the corneal pathology of MGK describe a vapor cup system that allows SM vapor injury, as opposed to a topical drop, but restricts the injury to the cornea and preserves conjunctiva and eyelids (McNutt et al., 2012; Milhorn et al., 2010). This mimics the battlefield type of injury but restricts it to the cornea only.

Another glass goggle model draws from established alkali injury models in rabbits using a filter paper soaked in NaOH placed on the cornea for a predetermined length of time to produce alkali injury (Anumanthan et al., 2018; Gronkiewicz et al., 2016). This method of injury uses a filter paper soaked in SM and mounted within a glass goggle attached over the rabbit eyes for several minutes, which produces a vapor injury similar to moderate to severe battlefield injuries (Kadar et al., 2009).

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MECHANISMS OF MGK INJURY AND PATHOPHYSIOLOGY

Ocular injury via SM injury has been extensively studied in both human corneas as well as in the aforementioned animal models. Due to the large aqueous-mucous interface between the cornea and the environment, the highly lipophilic SM is rapidly absorbed and reacts irreversibly with ocular tissues. The damage caused by SM is multifactorial, due to activation of a variety of mechanisms of oxidative damage, inflammation, cell death, and signaling pathways that ultimately lead to the clinical manifestation of acute injury and subsequent chronic or delayed-onset MGK depending on the course of disease. Figure 1 depicts the multifactorial mechanisms of damage caused by SM, including oxidative stress, angiogenesis, corneal inflammation and fibrosis, cytoskeleton and DNA damage with key cytokines, growth factors, and proteins involved in their pathogenesis. The general principles of corneal injury and corneal wound healing are induced, including induction of cyclooxygenase (COX)-1 and COX-2, transforming growth factor beta (TGF-β), activation of profibrotic genes, oxidative stress, DNA damage, cytoskeletal changes, angiogenesis, and production of fibronectin, however the precise mechanism responsible for MGK following SM exposure to the eye is still unclear and under investigation (Horwitz et al., 2019; Kamil and Mohan, 2020; Mohan et al., 2021; Tripathi et al., 2020).

ACUTE INJURY

Acute SM injury may be due to either DNA alkylation or due to free radical production via glutathione depletion, both of which ultimately result in cell death and tissue injury (Aasted et al., 1987; Borak and Sidell, 1992; Dahl et al., 1985; Etezad-Razavi et al., 2006; Laskin et al., 2010). SM forms highly reactive intermediates which can alkylate DNA and cause crosslinking primarily at the nitrogen residue of guanine (Wheeler, 1962). DNA alkylation leads to a complex cascade to cellular damage via inhibition of glycolysis, release of tissue proteases, and eventually cellular necrosis (Baradaran-Rafii et al., 2011; Solberg et al., 1997; Wheeler, 1962). There may also be delayed consequences unseen until cell populations that are quiescent at the time of exposure later become transcriptionally active and genotoxic damage is evident. Another proposed mechanism for acute SM injury is due to depletion of glutathione and excess free radical production resulting in cellular necrosis and acute tissue injury seen clinically (Aasted et al., 1987). Regardless of mechanism, histopathologic studies using rabbit models have demonstrated SM-induced corneal epithelial cell death and eosinophilic infiltration (Goswami et al., 2019; Kadar et al., 2009; McNutt et al., 2012). Epithelial-stromal separation leads to classically observed corneal edema (Kadar et al., 2009). Histopathology has also demonstrated stromal fibroblast necrosis, endothelial apoptosis and necrosis, resulting in corneal endothelial cell loss (Kadar et al., 2013a; McNutt et al., 2013; McNutt et al., 2016).

CHRONIC AND DELAYED INJURY

Chronic phase complications include persistent epithelial defects, limbal stem cell deficiency, corneal neovascularization, dry eye, and corneal opacification due to fibrosis as well as other long-term complications resulting in permanent discomfort and vision deficits or blindness (Etezad-Razavi et al., 2006; Ghabili et al., 2010; Ghasemi et al., 2009; Khateri

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et al., 2003; McNutt et al., 2012; Milhorn et al., 2010). While there may not be obvious inflammation, there is often vascular necrosis resulting in ischemia (Banin et al., 2003; Baradaran-Rafii et al., 2010).

In the delayed form of MGK, after a period of apparent resolution, the patient will once again begin to experience symptoms of photophobia, tearing, and foreign body sensation (Pleyer et al., 1999; Rajavi et al., 2017). Clinically, patients display corneal edema, corneal vascularization, opacification due to fibrosis or deposition of lipid and mineral, persistent epithelial defects, and dry eye (Baradaran-Rafii et al., 2011; Wolfe et al., 2019). Chronic and delayed MGK are clinically distinguishable but may represent a continuum of disease in terms of pathophysiologic changes.

Rabbit models of MGK have demonstrated persistent, unresolving corneal edema, suggesting that edema is an important component in the development of chronic SM injuries. Early studies showing abnormalities in endothelial cell density and morphology have been supported by more recent work in rabbit models (Kadar et al., 2013a; Lagali and Fagerholm, 2009; McNutt et al., 2013; Shohrati et al., 2007). Data from McNutt *et. al.* demonstrated loss of endothelial cells and failure of the corneal endothelial barrier function and correlated severity of endothelial damage to development of chronic MGK (McNutt et al., 2013). Due to the limited regenerative capacity of the human corneal endothelium and the importance of the endothelial cell pumps for maintaining deturgescence and corneal clarity, this is suggested to play an important role in the pathology of MGK (McNutt et al., 2016; Panahi et al., 2017b; Schmidt et al., 2016).

Previous studies have shown evidence of persistent inflammation affecting the cornea and anterior segment following SM exposure. Milhorn *et. al.* showed increases in interleukin (IL)-1β, tumor necrosis factor alpha (TNF-α), IL-6, IL8, matrix metalloproteinase (MMP)-2, and MMP-9 in aqueous humor of rabbits in the 16 weeks after a 2.5 min exposure to SM. These are all inflammatory mediators that can contribute to the continued damage seen in clinical patients (Milhorn et al., 2010). A study on pathogenesis of MGK injury demonstrated the presence of MMP-2 and MMP-9 in wounded corneas 7 weeks after SM exposure versus control corneas, as well as increased levels of pro-inflammatory mediators IL-1β, TNF-α, IL-6, and IL-8 in the aqueous humor at 1 and 7 weeks in rabbits after exposure (McNutt et al., 2012). Another group demonstrated elevations in corneal pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and IL-8 and macrophage chemotactic protein (MCP)-1 in limbal tissues of rabbits after a 4-minute exposure using a vapor model (Horwitz et al., 2018). Newer research in corneal mRNA levels after SM exposure has supported the involvement of pro-inflammatory mediators IL-6, IL-20, IL-22, IL-33, MMP-2 and MMP-9 (Horwitz et al., 2019).

Limbal epithelial stem cells (LESCs) reside primarily in the basal region of the limbus and are the main source of migrating new cells to produce daughter cells for normal homeostasis as well as to replace damaged cells in corneal injury (Baradaran-Rafii et al., 2010; Javadi et al., 2011). When a corneal injury occurs, LESCs are activated by a complex cascade of cytokines and inflammatory mediators to regenerate the damaged epithelium (Javadi et al., 2005). SM exposure is known to impair limbal vascular function as well as cause severe

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inflammation, which results in limbal stem cell deficiency (LSCD) (Javadi and Baradaran-Rafii, 2009; Javadi et al., 2011). LSCD may also be a manifestation of DNA alkylation and failure of transcription (Javadi et al., 2005).

Corneal neovascularization occurs in as many as 70% of MGK patients (Javadi et al., 2005; Khateri et al., 2003). In health, the cornea is avascular. In corneal injury and LSCD, neovascularization may be promoted. General mechanisms of corneal neovascularization are related to inflammatory mediators including, in particular, vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MPPs), particularly MMP-9 (Horwitz et al., 2018; Jafarinasab et al., 2010; Kadar et al., 2014; Lee and Chung, 2012; Sharma et al., 2010). Some of these mediators have been shown in some of the aforementioned studies to be present after exposure in rabbit models of SM exposure. Other experiments have localized VEGF and MMP-9 in the cornea in response to nitrogen mustard as well as SM exposure in rabbit exposure models (Tewari-Singh, 2014). Recent mRNA research using a rabbit model has also identified elevated levels of angiogenic MMPs, IL-20, IL-6, extracellular signal-regulated kinase (ERK)-5 signaling, and a number of growth factors including nerve growth factor, platelet-derived growth factor, hepatocyte growth factor, insulin-like growth factor, and fibroblast growth factor (Horwitz et al., 2019). This study highlighted for the first time some new pathways and signaling pathways potentially involved in induction of corneal vascularization in the delayed MGK pathology. The same study also showed downregulation of the anti-angiogenic glycoprotein, thrombospondin (TSP)-1, in clinically impaired rabbit eyes exposed to SM compared to non-impaired eyes (Horwitz et al., 2019).

In MGK, corneal opacification and vision impairment occur for multiple reasons. Corneal fibrosis develops when normal corneal wound healing results in transformation of keratocytes to fibroblasts and subsequently to myofibroblasts (Kanavi et al., 2010; Tripathi et al., 2020). Myofibroblast transformation occurs via the activation of numerous cytokines and inflammatory mediators, notably TGFβ−1, as well as others (Kanavi et al., 2010; Richter et al., 2006; Ruff et al., 2013). Other causes of corneal opacity in MGK include deposition of lipid and mineral in the corneal due to chronic inflammation and abnormal corneal vascularization which may leak lipid and amyloid (Mann and Pullinger, 1942; Milhorn et al., 2010; Razavi et al., 2012; Richter et al., 2006).

Dry eye symptoms are a common sequela of MGK, though the pathophysiology of dry eye development is not fully understood. Some suggested mechanisms of include damage to the goblet cells, lacrimal gland damage, neurotrophic dry eye, abnormalities of the sebaceous glands of the eyelids due to either direct damage by SM or as a consequence of severe blepharitis, and/or immunologic changes (Etezad-Razavi et al., 2006; Javadi et al., 2005; Razavi et al., 2012).

In addition to other histopathological findings, there is evidence in some studies of damage to the corneal nerves that persists for months after initial exposure in rabbit models (Kadar et al., 2009; Kadar et al., 2013b). One study showed evidence of Wallerian degeneration in exposed rabbits which continued for months (Kadar et al., 2009). Given the importance of corneal innervation on normal corneal maintenance and wound healing, this pathology may also contribute to MGK (Jafarinasab et al., 2010; Lagali and Fagerholm, 2009).

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TREAMENT MODALITIES

To date, no specific treatment is available to stop or reverse the effects of SM injury and no treatment prevents or reverses the signs associated with chronic and delayed MGK (Balali-Mood and Hefazi, 2005).

Treatment of acute SM injury is aimed at pain relief, reduction of absorption, anti-inflammatories, and prevention of infection (Balali-Mood and Hefazi, 2005; Panahi et al., 2017a; Rajavi et al., 2017; Wolfe et al., 2019). Early treatments include copious rinsing of the eyes as soon as exposure is realized as well as frequent application of topical lubricants (Safarinejad et al., 2001; Wolfe et al., 2019). Petroleum-based lubricating ointments are not recommended due the ability of SM to concentrate in the oily medium (Borak and Sidell, 1992). Symptomatic therapies for surface inflammation and anterior uveitis include topical and systemic corticosteroids, non-steroidal anti-inflammatories, and topical mydriatic agents (Solberg et al., 1997). Topical antiglaucoma medications are warranted in cases where intraocular pressure begins to rise. Antibiotic drops are used prophylactically to prevent infection (Murray and Volans, 1991). Dark glasses are often provided for patients due to photophobia (Dahl et al., 1985). There have been reports of amniotic membrane transplantation in acute injuries with bullae and erosions to help control inflammation and promote corneal healing while reducing scar formation (Balali-Mood and Hefazi, 2005). A newly published study examined the use of a combination topical therapeutic using ketorolac, Vorinostat, enalapril, and ascorbic acid administered twice daily to SM-exposed rabbit eyes *in vivo*. (Tripathi et al., 2020) Use of this combination drop for 3 days after exposure demonstrated improved Fantes scores, reduced corneal edema, and reduction in pro-fibrotic markers in treated eyes versus untreated SM-exposed eyes.

Management of chronic and delayed-onset MGK is challenging and aimed at improving quality of life and reducing discomfort depending on the patient's primary symptoms and clinical signs. It may include a combination of topical therapies to control inflammation and improve tear film, address secondary corneal infections, as well as surgical techniques to increase corneal support or replace limbal stem cells. New research is emerging as the pathophysiology of MGK is better understood (Feizi et al., 2013; Ghasemi et al., 2019; Gordon et al., 2010; Goswami et al., 2018; Naderi et al., 2019; Rajavi et al., 2017; Tewari-Singh et al., 2012). Treatment is generally dependent on each individual's clinical signs and response to therapy

Persistent epithelial defects (PED) may be managed medically or surgically depending on the patient and other contributing factors such as dry eye and limbal stem cell deficiency. Medical therapies may be as conservative as topical lubricants alone (Baradaran-Rafii et al., 2011). Topical antibiotics are often used to help prevent infection. Some reports have shown success in improving healing of PED with the use of autologous serum which contains growth factors and is theorized to promote normal corneal wound healing (Amitai et al., 2005; Giannaccare et al., 2017; Shtein et al., 2020). In severely injured eyes or eyes which are non-responsive to medical therapy, surgical therapy may be necessary. Severe cases may begin to experience dramatic corneal thinning. Tarsorrhaphy has been useful in protecting the corneal surface and preventing corneal thinning (Javadi and Baradaran-Rafii,

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2009). Amniotic membrane transplantation is also used to provide support, promote corneal healing, and reduce inflammation, but is not sufficient alone in cases of complete LSCD (Javadi and Baradaran-Rafii, 2009; Kadar et al., 2007). Additional promising regenerative therapies under investigation include using cultivated stem cells from sources such as bone marrow, adipose, umbilical cord, and orbital fat (Baradaran-Rafii et al., 2011; Gu et al., 2009; Lin et al., 2013; Panahi et al., 2017b). Other drugs have been researched such as thymosin β−4, a 43 amino acid polypeptide which promotes corneal wound healing when applied topically (Sosne et al., 2001). It has been suggested to be a possible antidote or therapeutic for SM exposure (Milhorn et al., 2010; Sosne et al., 2001).

Limbal stem cell deficiency is often currently managed surgically by stem cell transplantation (Coster et al., 1995; Tseng et al., 1998). Due to MGK generally being a bilateral condition by the nature of exposure, conjunctival limbal autografting presents unique challenges and is often not possible as it would be in other causes of LSCD (Javadi and Baradaran-Rafii, 2009; Shimazaki et al., 2004). Other transplantation techniques such as living-related conjunctival-limbal allograft and keratolimbal allografts have been reported to be possible in conjunction with immunosuppressive therapies to prevent graft rejections (Baradaran-Rafii et al., 2010; Jafarinasab et al., 2011; Javadi and Baradaran-Rafii, 2009; Shimazaki et al., 2004; Tseng et al., 1998).

Corneal neovascularization in MGK has been treated as in other similar conditions with topical anti-inflammatory medications including corticosteroids and non-steroidals (Amir et al., 2000; Javadi et al., 2005). Their use in combination has been shown to be more effective than as single agents. However, as the doses decrease, the corneal vascularization recurs (Kadar et al., 2009). In addition, there has been a proven association of long-term use of topical corticosteroids with development of glaucoma in people (Carnahan and Goldstein, 2000). Inhibitors of MMPs may be beneficial when initiated soon after SM exposure (Ghasemi et al., 2019). Several studies have suggested the benefit of topical doxycycline, a tetracycline with anti-inflammatory and MMP inhibitory activity, in prevention of neovascularization (Horwitz et al., 2014; Kadar et al., 2009; Tewari-Singh et al., 2012). One experimental model used doxycycline drops and hydrogels *in vivo* after SM exposure (Gordon et al., 2010). Their work showed good results in regard to doxycycline reducing edema and neovascularization (Gordon et al., 2010). Efficacy of compounds such as bevacizumab, an anti-VEGF antibody that can be applied topically have also been studied and showed some improvement in corneal neovascularization (Kadar et al., 2014). Other therapies such as fine-needle diathermy, photodynamic therapy, and alternative antiangiogenic medications have been shown to have varying levels of benefit in reducing the vascularization (Cowan et al., 1992; Panahi et al., 2017a; Panahi et al., 2017b). While not specifically studied in relation to SM exposure, ascorbic acid has been shown in multiple *in vivo* studies in rabbits to attenuate corneal neovascularization via inhibition of VEGF and may be useful in future therapeutic modalities (Lee and Chung, 2012; Levinson et al., 1976; Pfister et al., 1988).

Clinical signs of dry eye significantly contribute to chronic discomfort. Frequent use of topical tear replacement drops and gels is often advocated (Balali-Mood and Hefazi, 2006). Traditional dry eye treatments such as punctal plugs are also used commonly (Rajavi et

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al., 2017). Topical anti-inflammatory steroids may benefit patients with dry eye, however, alternatives such as topical cyclosporine have also been shown to be beneficial and have fewer side effects (Carnahan and Goldstein, 2000). Cyclosporine is a calcineurin inhibitor and is approved for the treatment of dry eye in people as well as animals and is widely available (Jadidi et al., 2015). In addition to improving tear production and tear film quality it also modulates the immune response by inhibiting T-cells via blockage of the IL-2 signaling pathway and may contribute to reduction of inflammation (Jadidi et al., 2014). Emerging therapies for dry eye may also have promise in for patients with MGK-related signs. Tacrolimus is a macrolide with immunomodulatory properties and has been shown to be efficacious in treatment of dry eye due to Sjogren's syndrome and immune-mediated causes (Moscovici et al., 2012; Moscovici et al., 2015; Panahi et al., 2017b). It is commonly used in the treatment of veterinary patients with dry eye (Berdoulay et al., 2005; Hendrix et al., 2011; Radziejewski and Balicki, 2016; Zulim et al., 2018). Tacrolimus may also be effective topically for SM-induced dry eye, although more research for this application is warranted (Panahi et al., 2017a).

Corneal opacification due to MGK is often treated surgically with corneal transplantation in an attempt to restore corneal clarity and improve vision. Depth of the opacity dictates the need for a lamellar keratoplasty versus a penetrating keratoplasty (Jafarinasab et al., 2011; Javadi et al., 2007). Patients undergoing penetrating keratoplasty due to MGK are at high risk for graft rejection due to the severity LSCD, dry eye, corneal vascularization, and damage to corneal innervation which impairs normal healing mechanisms (Baradaran-Rafii et al., 2010; Feizi et al., 2013). Attempts to manage the other chronic components of the condition via medical or surgical therapies prior to corneal transplantation is important to improve success. One study reported rates of long-term graft survival for penetrating keratoplasty of 39% versus 90.3% for lamellar keratoplasty (Javadi et al., 2011). Even after grafting, patients may still experience recurrence of opacification. As more information emerges regarding effects of SM exposure on the corneal endothelium, endothelial transplant therapy may be warranted, though no reports of this technique specific to SM-exposed patients currently exist (Dabrowska et al., 1996; McNutt et al., 2013; McNutt et al., 2016).

Other medical therapies that have been investigated to reduce ocular inflammation and improve ocular outcomes including calcium channel blockers (diltiazem), colchicine, glutathione, n-acetylcysteine, silbinin, and others (Amir et al., 2000; Balszuweit et al., 2013; Cowan et al., 1992; Gonzalez et al., 1995; Laskin et al., 2010; Mazumder et al., 1998; Tewari-Singh et al., 2012). Diltiazem has been reported to reduce ocular inflammation after exposure (Gonzalez et al., 1995; Mazumder et al., 1998). Silbinin is proposed to be an antidote to SM or nitrogen mustard exposure (Balszuweit et al., 2013; Tewari-Singh et al., 2012). Glutathione and its precursor n-acetylcysteine may reduce oxidative stress and thus reduce the effects of SM on the ocular tissues (Cowan et al., 1992).

ONGOING STUDIES AND FUTURE DIRECTIONS

In spite of nearly a century of study, the mechanism of development of latent or chronic MGK is still not fully understood, signifying the importance of ongoing research to combat this toxin. Current research is aimed at development of new ocular SM injury models, as

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well as further study of the tissues affected by SM injury as well as the tissues, we know are imperative for corneal function.

As progress is made in the study of corneal wound healing models and our understanding of how to mitigate corneal fibrosis and corneal neovascularization, these therapies may also be applicable to the treatment of MGK. As SM exposure remains a risk on both modern battlefields and as a bioweapon, the search for an effective therapy is ongoing. The use of a multimodal topical therapeutic drop is being investigated in our laboratory to target several of the mechanisms that are thought to contribute to MGK development.

ABBREVIATIONS

SM	Sulfur mustard
MGK	mustard gas keratopathy
TGF-β	transforming growth factor beta
COX	cyclooxygenase
VEGF	vascular endothelial growth factor
IL	interleukin
MMP	matrix metalloproteinases
MCP-1	macrophage chemotactic protein-1
LESCs	limbal epithelial stem cells
LSCD	limbal stem cell deficiency
ERK5	extracellular signal-regulated kinase
TSP-1	thrombospondin-1
PED	Persistent epithelial defects
DNA	deoxyribonucleic acid
ROS	reactive oxygen species
GSH	glutathione
LPO	lipid peroxidation
PARP	poly ADP-ribose polymerase
AP1	activator protein 1
bFGF	basic fibroblast growth factor

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Highlights

- Mustard gas is a highly toxic alkylating agent that rapidly penetrates into ocular tissues.
- Sulfur mustard gas exposure to eye causes multiple ophthalmic manifestations including persistent corneal epithelial defects, corneal neovascularization, corneal opacification, dry eye, and limbal stem cell deficiency.
- MGK involves unique acute, chronic, and delayed ocular manifestations that can persist for months and years post-exposure.

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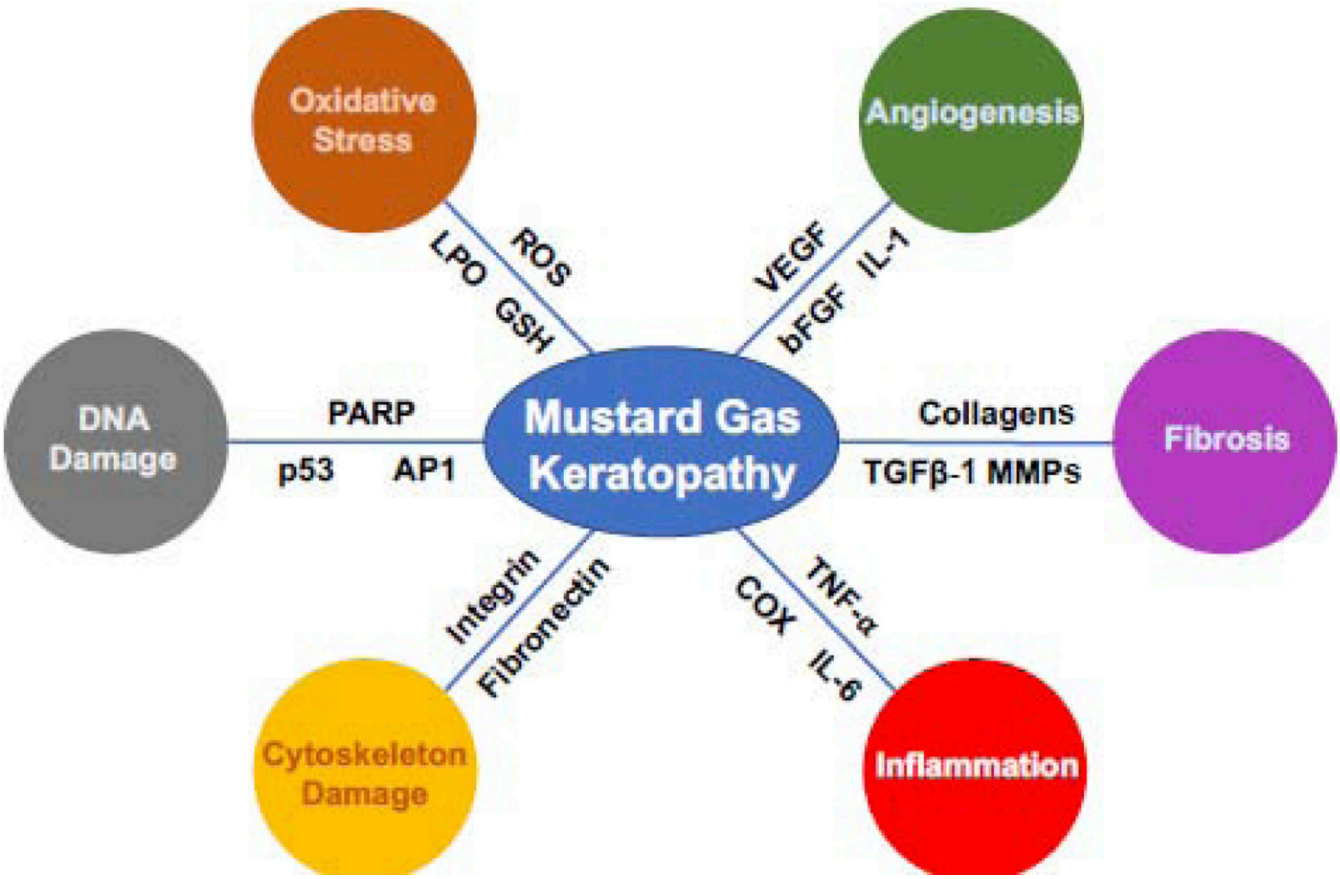

Fig. 1.

Schematic showing processes and factors associated with pathogenesis and mechanisms during sulfur mustard toxicity and MGK.