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Contributions of tissue-specific pathologies to corneal injuries following exposure to SM vapor

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Abstract

Corneal injuries resulting from ocular exposure to sulfur mustard (SM) vapor are the most prevalent chemical warfare injury. Ocular exposures exhibit three distinct, dose-dependent clinical trajectories: complete injury resolution, immediate transition to a chronic injury, or apparent recovery followed by the subsequent development of persistent ocular manifestations. These latter two trajectories include a constellation of corneal symptoms that are collectively known as mustard gas keratopathy (MGK). The etiology of MGK is not understood. Here we synthesize recent findings from *in vivo* rabbit SM vapor studies suggesting that tissue-specific damage during the acute injury can decrement the regenerative capacities of corneal endothelium and limbal stem cells, thereby predisposing the cornea to the chronic or delayed forms of MGK. This hypothesis not only provides a mechanism to explain the acute and MGK injuries, but also identifies novel therapeutic modalities to mitigate or eliminate the acute and long-term consequences of ocular exposure to SM vapor.

Keywords

sulfur mustard; cornea; corneal epithelium; limbal stem cell

Introduction

Blister agents are highly effective chemical weapons that cause vesicating burns to exposed skin, eyes, and respiratory tract, with lethality occurring at high doses. The bifunctional alkylating chemical warfare agent (CWA) sulfur mustard (SM; $C_4H_8Cl_2S$) is the prototypical blister agent. SM has proven to be a potent and versatile chemical threat, and battlefield use of SM is responsible for more than 80% of chemical casualties over the last century.^{1–5} Although not an exceptionally lethal CWA, SM causes incapacitating and painful injuries that require prolonged rehabilitation. The physicochemical properties of SM and limited clinical options for SM casualties have made it the most effective chemical weapon in history, earning it the moniker “king of battlefield gases.”¹

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Conflicts of interest

The authors declare no conflicts of interest.

The eye is the most sensitive organ to SM injury, and more than 90% of SM casualties present with ocular comorbidities.^{5,6} Although mild exposures typically resolve without long-term complications, moderate-to-severe exposures involve a prolonged rehabilitation period during which victims are visually disabled.^{7–9} In some cases, survivors of severe exposures develop persistent corneal manifestations ranging from chronic ocular discomfort to advanced corneal disease characterized by progressive degeneration and impairment or loss of vision.^{7–9} These persistent manifestations are collectively referred to as mustard gas keratopathy (MGK). Despite a century of research, the etiologies of the acute and persistent ocular manifestations of SM exposure are unknown, and the ocular injury has proven refractory to therapeutic efforts. Given the large numbers of current MGK victims, as well as the reported use of SM in ongoing conflicts, understanding the pathophysiological mechanisms involved in ocular SM injury is critically important to developing comprehensive treatment strategies that extend beyond supportive care. Here we review key aspects of corneal SM injury, with particular emphasis on newly described tissue-specific pathologies that shed light on long-lasting questions about disease progression.

Molecular toxicology of SM injury

Pure sulfur mustard is a dense, viscous liquid that is poorly soluble in water but highly soluble in organic solvents and lipids.¹⁰ Because of its high lipid solubility, SM is rapidly absorbed into biological tissues, where it cyclizes to form highly reactive, cyclic episulfonium intermediates, which can alkylate (i.e., replace a proton with an alkyl group) nucleophilic moieties of lipids, nucleic acids, and proteins (Fig. 1).¹¹ This intramolecular cyclization occurs independently on both arms of the molecule, and therefore SM can cross-link two nucleophilic sites within or between molecules. Because episulfonium ions interact with a wide variety of biological macromolecules, the cellular consequences of alkylation are complex and multifactorial. It is currently believed that alkylation and cross-linking of guanine nucleotides are the principal injury modality, activating futile DNA repair mechanisms, blocking DNA synthesis, and disrupting gene expression.¹² The resulting cellular damage contributes to a variety of cytotoxic and genotoxic processes.⁶ Genotoxic damage would be expected to have immediate consequences to metabolically active or proliferating cell populations. Alternatively, cell populations that are quiescent at the time of exposure may not exhibit toxicity until they become transcriptionally active or begin proliferating.

Ocular injury is typically caused by exposure to SM vapor

The liquid form of SM is stable for weeks at room temperature, hydrolyzes slowly in water and has relatively low volatility (Fig. 1).¹³ Because of these physicochemical characteristics, the most common strategy during World War I was to deploy SM liquid at night, when cooler temperatures extended environment persistence.¹³ Troop exposures primarily occurred after sunrise, as rising temperatures produced SM vapor, with elevated exposure risks occurring in poorly ventilated areas such as trenches. Since SM has an asymptomatic latent period of 2–12 h, victims often suffered large cumulative doses because they were unaware of ongoing exposure.⁵ In contrast to the use of SM in World War I, Iraqi deployment of SM against Iranian troops and civilians predominantly occurred during the

day, producing an immediate vapor threat due to the high temperatures and low humidity.¹⁰ Because of the relationship between physicochemical characteristics and tactical deployment of SM, vapor exposure accounts for over 80% of SM casualties in these two conflicts.^{5,13}

Clinical manifestations of ocular SM vapor exposure

The elevated metabolic rate of corneal epithelial cells and the large aqueous–mucous interface between the corneal surface and the tear film render the cornea highly sensitive to SM injury.^{5,6,14} The spectrum of ocular injury is primarily determined by exposure time and concentration, with acute symptoms ranging from mild conjunctivitis to corneal lesions and loss of vision.⁶ Human clinical studies conducted in the mid-20th century have provided rigorous concentration:time (Ct) data for ocular vapor SM injury.^{15–19} On the basis of these experiments, the minimum effective dose was determined to be 5–30 mg·min/m³; the minimum incapacitating dose was determined to be 60 mg·min/m³; and the median incapacitating dose (IC₅₀) was determined to be 100–200 mg·min/m³.^{9,20–22} For reference, the typical World War I battlefield SM vapor concentration is estimated to have been 19–33 mg/m³.⁸ At these concentrations, exposure durations as short as 3 min would exceed the minimum incapacitating dose, whereas 5 min would exceed the IC₅₀.

Ocular SM injuries follow one of three clinical trajectories (classes I–III), which correlate to exposure dose and are distinguished by the severity of the symptoms and the recovery time (Table 1).^{23–25} While complete injury resolution is the most common outcome for class I/II exposures, class III exposures often involve persistent corneal manifestations that are refractory to treatment. The collective symptomology of these manifestations is known as MGK and can develop directly from the acute injury (the chronic form) or emerge after a latent period ranging from 0.5 to 40 years (the late-onset form).^{8,11} The etiology of either form of MGK is unknown. In large-cohort, longitudinal clinical studies of survivors of class III exposures, 35–95% were diagnosed with mild symptoms of MGK, including photophobia, tearing, foreign body sensation, corneal neovascularization, and corneal erosions.^{7–9} Severe symptoms were identified in 0.5–1.5%, featuring advanced corneal disease characterized by progressive corneal degeneration and impairment or loss of vision.^{7–9,26} Analyses of human MGK corneas have revealed histological evidence consistent with the clinical manifestations, including chronic keratitis, corneal thinning and ulceration, neovascularization, and corneal degeneration.^{27–29} Collectively, these studies suggest a persistent disease that is beyond the healing capacity of the cornea.

SM reactivity within biological tissues is rapid and irreversible, and consequently current treatment strategies are focused on mitigating pathological host responses and promoting healing.³⁰ While mild forms of MGK can usually be managed symptomatically, patients still suffer from a significantly lower quality of life as a consequence of their chronic injuries.^{31,32} More severe cases of MGK are progressive and difficult to manage clinically. Surgical interventions, such as corneal keratoplasty and limbal stem cell transplants, have had mixed outcomes, with successes largely restricted to cases with mild or moderate MGK symptoms.^{27,29,33,34} As discussed below, the corneal response to SM exposure involves injury processes that occur on different time scales in multiple corneal tissues, and therefore we

believe that development of effective treatment strategies is likely to require improved understanding of specific pathophysiological mechanisms.

Tissue-specific aspects of SM injury: overview

While early therapeutic approaches have focused on preventing epithelial vesication and reducing corneal edema using anti-inflammatory drugs, the effects of SM exposure on other corneal tissues have been largely ignored. Recent studies in vapor-exposed rabbit eyes have identified several new tissue-specific pathologies that appear to explicate many aspects of SM injury.^{35,36} Understanding the importance of these pathologies to SM injury progression requires an appreciation of the functional and regenerative capacities of corneal tissues. The cornea is responsible for two-thirds of the dioptric power of the eye.³⁷ The majority of this refractory power comes from the corneal stroma, which is an avascular tissue composed of tightly packed collagen lamella that makes up 90% of the corneal volume (Fig. 2).³⁸ Uniform fibril packing is critical in rendering the stroma transparent to light, and functional integrity of the stroma is highly dependent on maintenance of a deturgescent state.³⁷ If corneal hydration increases by more than 5% above physiologic levels, vision becomes significantly impaired.³⁹ The degree of stromal hydration is primarily controlled by two tissues: the corneal epithelium at the anterior margin and the corneal endothelium at the posterior margin.³⁹ Disruption of either cell barrier allows fluid entry into the stroma, rapidly eliciting stromal edema, disorganizing the collagen lamella, and disrupting vision. Chronic edema also elicits secondary keratopathies that can cause corneal degeneration and loss of vision.⁴⁰ Recent reports have demonstrated SM-induced decrements in regenerative capacities of the corneal epithelium and endothelium, therefore impairing the ability of these two tissues to maintain corneal hydrostasis.^{35,41} Here we discuss the implications of these injuries to corneal function and disease progression in SM-exposed eyes.

Animal models of ocular SM injury

Animal models have been used to study pathogenesis of acute and persistent SM ocular injuries for nearly a century.^{6,22,42} One of the most productive models has been *in vivo* vapor exposure in rabbits. The rabbit cornea is structurally similar to the human cornea, and although rabbits are 4-fold less sensitive to ocular SM injury, rabbit and human eyes exhibit nearly identical lesions at functionally equivalent doses.^{20,22} Rabbit models of vapor SM exposure undergo dose-dependent, multiphasic corneal injuries with sequelae similar to those observed in human victims.^{43–45} These studies have revealed pathophysiological mechanisms associated with the corneal SM injuries and provided a test platform for candidate therapeutics. In our version of the ocular SM exposure model, SM vapor is applied to the corneal surface via a saturated vapor cap, with the exposure dose determined by the duration of exposure.⁴⁴ An o-ring is used to restrict SM exposure to the area of the vapor cap, eliminating pericorneal lesions resulting from lateral diffusion. We have used this vapor exposure model to describe dose- and time-dependent effects of SM on the structure, biochemistry, and function of corneal tissues.^{35,44–46} Briefly, within 24 h of exposure, corneas develop acute lesions characterized by vesication of the corneal epithelium, stromal keratocytosis, corneal edema, and corneal endothelial cell loss. The corneal epithelium undergoes a robust healing response to regenerate an impermeable epithelial cap within 5

days (Fig. 3A and 3B), which develops into an immature, stratified epithelium with rudimentary hemidesmosomal adhesions by 7 days. Corneas continue to improve through 2 weeks (the so-called quiescent phase), with few clinical symptoms other than corneal opacity and lingering edema. Corneas then either heal or transition directly to a progressive injury that appears to correspond to the chronic form of MGK. Resolving corneas undergo a steady decrease in corneal thickness, reaching baseline levels by 6 weeks; a time frame similar to class II or IIIa injuries in humans (Fig. 3C).^{43,45} In contrast, the failure of corneal edema to resolve at 3 weeks and beyond is the earliest clinical marker of corneas that will develop chronic MGK. Histopathological and structural markers of MGK are rapidly expressed from 2–4 weeks in these corneas and include recurring basal epithelial cell cytotoxicity, basement membrane zone degeneration, loss of limbal epithelial stem cells (LESCs), inflammatory cell infiltration, elevation of cytokines, persistent endothelial failure, delayed in-migration of keratocytes, stromal degeneration, and redundant deposition of basement membrane components caused by cyclical attempts to regenerate the epithelium and endothelium (Fig. 3D).^{36,43,45–47} Secondary clinical symptoms such as epithelial bullae formation, recurring corneal erosions, neovascularization, and limbal stem cell disorder (LSCD) subsequently develop, preventing stable repair of the ocular surface.⁴⁸ Postexposure, steroid-based anti-inflammatory treatment protocols reduce and postpone the appearance of the late injury.^{48–50} However, they do not prevent MGK, suggesting that anti-inflammatories fail to treat the pathologies responsible for MGK onset. It should be noted that an animal model of late-onset MGK is currently not available, limiting the ability to study the specific pathophysiology associated with the delayed manifestation of MGK.

Tissue-specific aspects of SM injury: effects on the corneal limbal epithelium

The corneal epithelium is composed of 5–7 stratified cell layers and presents a highly regenerative, impermeable barrier between the external environment and the corneal stroma.⁵¹ Homeostasis of the corneal epithelium primarily relies on a small population of limbal epithelial stem cells (LESCs) located in the basal region of the limbus (Fig. 2).⁵² In healthy corneas, LESCs slowly cycle to produce daughter transient amplifying cells (TACs), which migrate laterally into the basal layer of the corneal epithelium and proliferate to compensate for exfoliation of superficial corneal epithelial cells. Under conditions of corneal injury, increased numbers of LESCs are activated to enhance regeneration of damaged epithelium. The extensive regenerative capacity of the corneal limbus is illustrated by its ability to restore a stratified epithelial cap within days after denudation of over 50% of the corneal surface.⁴⁵ Although the limbal niche can provide a robust regenerative capacity, it is very sensitive to environmental and chemical insult. Pathological conditions, such as persistent inflammation or impaired limbal vascular function (both acute manifestations of SM exposure), can disrupt niche function, causing LESC loss and destabilizing the corneal epithelium.^{40,53} The resulting corneal disease, LSCD, manifests with symptoms associated with MGK, such as neovascularization, chronic inflammation, recurrent corneal erosions, keratitis, corneal ulceration, and stromal scarring.^{54,55} In the rabbit vapor exposure model, LESCs have been found to remain viable and are activated during acute SM injury.⁴⁸ However, molecular markers of LESC viability are subsequently reduced 2–4 weeks after

exposure, coincident with onset of MGK symptoms. Collectively, these studies suggest that limbal niche failure is a delayed aspect of ocular SM injury. However, it is unclear whether LESC loss emerges secondary to the onset of MGK or is a latent consequence of genotoxic injury that is progressively expressed upon LESC activation during repair of the acute corneal epithelial lesion.

Tissue-specific aspects of SM injury: effects on the corneal endothelium

The corneal endothelium is a flattened monolayer of cells at the posterior margin of the stroma (Fig. 2). The endothelium presents a semipermeable barrier between the aqueous humor (AH) and stroma that maintains the cornea in a dehydrated state necessary for optical transparency. Corneal endothelial cells (CECs) are postmitotic, and CEC loss is compensated for by migration and enlargement of adjacent CECs.^{56,57} Once endothelial cell density falls below the threshold level required for barrier function, the endothelium can no longer maintain stromal deturgescence. The resulting chronic edema disrupts vision and elicits secondary keratopathies, such as anterior segment inflammation, epithelial bullae, and LSCD.^{40,58} Although frank endothelial cytotoxicity is an uncommon clinical morbidity, diseases involving significant loss of CECs, such as aphakic bullous keratopathy, produce symptoms that are strikingly similar to MGK.⁵⁹ Because of the limited regenerative capacity of the corneal endothelium, while minor endothelial lesions can be repaired, large lesions may either not be repairable or, alternatively, predispose the cornea to long-term complications resulting from reduced endothelial cell density.

Several clinical findings suggest that ocular exposure to SM vapor results in corneal endothelial injury. First, early studies reported the development of uveitis in casualties of class III exposures, indicating that SM vapor penetrates through the cornea to react with tissues in the anterior chamber.^{22,23} These findings were corroborated in animal studies demonstrating that radiolabeled SM vapor applied to the corneal surface accumulates in the anterior chamber within minutes.^{60,61} Second, human MGK corneas exhibit histological evidence of reduced endothelial cell density with increased variability in CEC size and morphology.⁶² These correlations led us to hypothesize that ocular SM exposure can cause acute endothelial toxicity. To test this hypothesis, we evaluated the functional and structural integrity of rabbit corneal endothelia following vapor SM exposure. Using methods such as electron microscopy, *in vivo* confocal microscopy, assessment of barrier function, and immunocytochemistry, we demonstrated frank loss of CECs and failure of the endothelial barrier at 24 h after exposure (Fig. 3D).³⁵ Furthermore, whereas resolved corneas had a healthy-appearing endothelium, MGK corneas exhibited severe endothelial pathologies characterized by persistent CEC cytotoxicity, abnormal cell morphologies, and diffusive thickening of the endothelial layer.³⁵ Notably, deposition of fibrous membranes is a common phenomenon in pathological conditions involving endothelial-to-mesenchymal cell transitions, suggesting irreversible failure of the endothelium.^{63,64} To better understand the causal relationship between endothelial injury and MGK, we compared the effects of exposure area on the likelihood of developing chronic MGK. At equivalent doses, the likelihood of chronic MGK onset was a function of the extent of the endothelial lesion, suggesting that inability to repair the endothelial barrier may be one cause of chronic MGK (manuscript in preparation). Collectively, our data demonstrate that SM vapor exposure can

result in gross lesions in the rabbit corneal endothelium and furthermore suggest that the inability to repair the endothelial barrier prolongs the acute lesion and contributes to chronic MGK onset.

Integrating tissue-specific pathologies into a candidate model of ocular SM injury progression

These data suggest that the distinctive symptoms of ocular SM exposure may result from injury processes that operate on different time scales in multiple corneal tissues. We propose that SM-induced reductions in the regenerative capacity of two specific tissues—corneal endothelium and the limbal stem cell niche—may explain the relationship between SM dose, severity of acute injury, and likelihood of MGK onset (Fig. 4). In this model, the extent of the endothelial lesion is a principal determinant of the severity of the acute SM injury. Furthermore, the efficiency of endothelial repair will dictate whether corneas resolve or develop one of the two forms of MGK. This conceptual framework allows us to speculate about dose-dependent injury mechanisms involved in corneal responses to SM exposure. For example, low-dose exposures (e.g., below $100 \text{ mg}\cdot\text{min}/\text{m}^3$) result in minor injuries to the conjunctiva and corneal epithelium that rapidly heal without long-term complications. As SM doses increase, so does the probability of direct injury to the endothelium. If the endothelial lesion is relatively small, then the endothelial barrier can be rapidly repaired, corneal edema will resolve and the cornea will begin to heal (injury resolution). Alternatively, if the endothelial lesion is sufficiently large to cause protracted edema before ultimately healing, secondary keratopathies, such as neovascularization or LSCD, develop that can have a delayed effect on corneal integrity. These corneas will have a high chance of manifesting late-onset MGK, as described below. Finally, if the endothelial injury proves too profound to repair, the acute injury will transition directly to chronic MGK, involving persistent edema through the posterior margin, inflammatory responses in the stroma, and secondary damage in other corneal tissues.

This model posits two potential mechanisms to trigger emergence of the late-onset form of MGK: progressive endothelial loss and damage to the limbal niche. Both mechanisms result from reductions in the long-term ability of corneal tissues to maintain homeostasis. In healthy adults, the reserve CEC capacity is sufficient to compensate for age-related CEC losses throughout the human life span.⁶⁵ In contrast, an injury-depleted corneal endothelium has a lower tolerance for CEC loss, such that normally insignificant declines in CEC numbers (e.g., as a natural consequence of aging) may reduce endothelial density below threshold levels, triggering persistent edema and eliciting symptoms of MGK. While there is histologic evidence of CEC loss in human MGK corneas, there is currently no direct evidence of endothelial failure.⁶² However, a potential role for endothelial cells in SM ocular injury has only been recently described, and therefore endothelial contributions to late-onset MGK have not yet been rigorously studied. An alternative explanation is that endothelial injury plays a direct role in the acute injury and chronic MGK, but contributes indirectly to late-onset MGK by eliciting secondary injury to the limbal niche as a result of prolonged edema during the acute injury, as discussed below.

A second possible mechanism of late-onset MGK is LESC depletion caused by direct or indirect SM injury to the limbal niche. This can occur in response to prolonged corneal edema or as a result of genotoxic injury to quiescent LSCs, which result in delayed cell death following activation.^{40,58} In either case, as the remaining LSCs are naturally depleted over time, the regenerative capacity of the limbus becomes compromised, destabilizing the corneal epithelium and eliciting a wide range of secondary keratopathies. In clinical cohort studies, 41–81% of MGK patients were diagnosed with LSCD.^{7,34} In some experimental cases, limbal transplants or lamellar keratoplasty has successfully treated ocular surface instabilities, although in both cases the likelihood of success was inversely related to the severity of corneal symptoms.^{34,66} However, as described above, it is unclear whether LSCD is causative of MGK or occurs secondary to MGK onset.

Finally, a third possibility is that late-onset MGK may result from either pathology. For example, direct endothelial failure may provide the etiological basis for the most severe cases of late-onset MGK, such as those involving complete loss of vision, whereas LSCD may be responsible for less severe instances of MGK primarily characterized by peripheral corneal symptoms.

Ongoing studies and future challenges

Ocular injuries resulting from SM exposure remain poorly understood, despite nearly a century of study. Here we integrate recent data to synthesize a new model of ocular SM injury that accommodates what is known about tissues that are (1) injured by SM vapor and (2) critically important to corneal function. This model proposes that acute injury progression and MGK onset are driven by decremented regenerative capacities of the corneal endothelium and limbal stem cell niche. These tissue-specific pathologies are consistent with clinical and histological observations in human and rabbit MGK corneas and offer physiologically plausible mechanisms to explain the average latency of 20 years between SM exposure and development of late-onset MGK.^{62,67} Although there is broad clinical support for a correlation between LSCD and MGK in human corneas, little effort has been devoted to evaluating endothelial injury during the acute injury or MGK. Establishing a causal relationship between specific corneal pathologies and SM exposure is further complicated by the fact that most casualties are decades beyond the point of exposure. Nonetheless, the possibility of tissue-specific contributions to corneal pathogenesis has important implications for SM therapeutic research, particularly with regard to endothelial lesions. First, the failure to address endothelial injury may explain why candidate therapies targeting the anterior cornea have had limited efficacy in preventing MGK. Second, if the extent of endothelial injury correlates with the likelihood of MGK onset, then quantitation of endothelial toxicity may offer a method to distinguish between eyes that will resolve and those at risk of developing MGK. Third, if SM causes endothelial injury, then toxicity may also occur in more posterior ocular tissues. Finally, early clinical interventions to restore endothelial function may mitigate or prevent the appearance of MGK.

Although there are still open questions regarding the extent to which endothelial cytotoxicity and LSCD contribute to SM ocular injury, it is encouraging that mechanistic studies have

moved beyond the acute effects of SM on the corneal epithelium. While this review does not address potential involvement of other corneal tissues to ocular SM injury, it may be that cells such as corneal nerves and keratocytes play a critical role in mediating injury responses. We hope that this review will positively motivate efforts to study the potential involvement of other tissues in SM injury as well as to identify novel therapeutic modalities that target these pathologies. Finally, we also believe that it is critically important to gain immediate and sustained access to recent SM casualties for evaluation with state-of-the-art scientific and medical equipment, something that currently is not feasible.

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Figure 1.

Physicochemical properties of SM. (Left) In aqueous environments, SM cyclizes with the loss of Cl⁻ to form a highly reactive episulfonium intermediate on either arm that alkylates biological macromolecules or undergoes hydrolysis. Nucleophilic reactive groups on biological macromolecules (:R) include amine, carboxyl, sulfhydryl, and imidazole moieties on lipids, DNA, RNA, and proteins. (Right) Summary table of physicochemical properties of pure SM.^{5,10}

Figure 2.

Overview of corneal structure. (Left) Cross section of ocular globe illustrating key tissues involved in vision. (Right) Magnified view demonstrating structure of the cornea (inset a) and corneal-limbal junction (inset b). Limbal epithelial stem cells (green) are located at the base of the limbal epithelium.

Figure 3.

Clinical and ultrastructural characterization of acute and MGK injuries in rabbit class III SM vapor exposures. (A) Fluorescein uptake assay demonstrating acute and recurrent epithelial lesions over 56 days. Note recurrence between 14 and 21 days after exposure, indicative of chronic MGK onset.⁴⁵ (B) Planimetric area of fluorescein uptake measured over 56 days after SM exposure ($n = 25$).⁴⁵ (C) Pachymetry measurements of corneal edema over 56 days after SM vapor exposure ($n = 56$) in sham-exposed ($n = 10$), MGK ($n = 56$) and resolved ($n = 10$) corneas.⁴⁵ Note that edema in MGK and resolved cornea are significantly different starting at 14 days. (D) Representative transmission electron microscopy of epithelium (top panels, scale bar = 2 μm), endothelium (middle panels, scale bar = 2 μm) and *en face* scanning electron microscopy of endothelium (bottom panels, scale bar = 10 μm) in sham-exposed corneas, 1-day corneas, 8-week resolved corneas, and 8-week chronic MGK corneas.^{35,46} Acute cytotoxicity of epithelial and endothelial cells results in denuded basement membranes at 1 day. MGK corneas exhibit ongoing cytotoxicity (*) in the epithelium and endothelium, epithelial bullae formation, retrocorneal fibrous membrane (RCFM) deposition, and loss of hexagonal endothelium morphology. In contrast, resolved corneas are structurally indistinguishable from sham-exposed corneas. bc, basal epithelial cell; wc, wing cell; ec, endothelial cell.

Figure 4.

Candidate model of class III corneal vapor SM injury progression. At sufficiently high SM vapor doses, healthy corneas (1) develop acute cytotoxicity and vesication in the epithelium and endothelium (2). Since the epithelium re-establishes an impermeable cap within days, the subsequent injury trajectory will be determined by the severity of the endothelial lesion (3). If the endothelial lesion is minor, the endothelium heals quickly and corneal edema resolves with no long-term consequences (3.a). If the endothelial lesion is moderate, recovery is delayed (3.b) and additional damage may be incurred to the limbal niche. The reduced regenerative capacity in the endothelium and/or limbal niche predisposes the cornea to late-onset MGK. Finally, if the endothelial lesion is so severe that an endothelial barrier cannot be restored, the chronic form of MGK develops (3.c).

Summary of ocular manifestations, recovery times and historical prevalence of ocular SM vapor exposures^{8,23,26,68}

Table 1.

Injury class	SM dose (mg·min/m ³)	Characteristic ocular manifestations	Symptomatic recovery	Historical prevalence
Class I	5–100	Conjunctival injection and ocular irritation, without lacrimation, blepharospasm, or photophobia. Symptoms appear 12–24 h after exposure.	1–2 weeks	75%
Class II	100–200	Above symptoms plus mild corneal involvement, such as corneal edema and epithelial vesication. Symptoms appear 6–12 h after exposure, including edema and epithelial erosions.	2–6 weeks	15%
Class III	> 200	Severe ocular pain, cornea clouding and impaired vision, blepharospasm, uveitis, limbal necrosis, chemosis, corneal erosions and corneal edema with possibility of long-term manifestations. At high doses, permanent visual impairment is likely. Symptoms appear within 2 h.	Months to never	10%