Dosing frequency-dependent effect of dexamethasone treatment on sulfur mustard-induced corneal injuries in rabbit ocular in-vivo model

Neha Mishra[1], Rama Kant[1], Kushal Kandhari[1], Neera Tewari-Singh[2], Poojya Anantharam[3], Claire R. Croutch[3]
Mina B. Pantcheva[1], Jonathan M. Petrash[1], Chapla Agarwal[1], Rajesh Agarwal[1]
[1]University of Colorado, Anschutz Medical Campus, Aurora, CO, USA; [2]Michigan State University, East Lansing, MI, USA; [3]MRIGlobal, Kansas City, MO, USA.

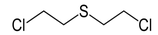
4095

Abstract

Sulfur mustard (SM) is the most infamous vesicating agent, extensively used in warfare. Eyes, due to their high-water content and physiological milieu, are most susceptible to SM-induced injuries, depending on A the route, duration, and dosage of exposure. Ocular SM injuries include irritation, redness, inflammation, fibrosis, epithelial degradation, blurred vision, partial/complete blindness. Targeted and effective countermeasures against SM-ocular exposure are unavailable and warranted in case of a terrorist activity or leakage from stockpiles. Dexamethasone (DEX), an FDA approved potent corticosteroid with documented anti-inflammatory activities, could be an effective treatment modality. Thus, we assessed the efficacy of DEX (0.1%) administration beginning at 2h post SM-exposure and then either every 8 or 12h thereafter for 28 days post SM exposure, in reducing corneal injuries in rabbit ocular *in-vivo* model. The rabbits were divided into two groups: Group I (right eye, SM exposed and left eye, control) and Group II (both eye SM exposed; right eye, DEX 12h and left eye, DEX 8h treatments). The parameters assessed were clinical (corneal opacity, ulceration, and neovascularization) at day 14, 28, 42, and 56, biological (epithelial degradation, epithelial-stromal separation, blood vessels density, and inflammatory cell count) and molecular (COX-2, MMP-9, and VEGF expression) at day 28, 42, and 56 post SM exposure. Results indicated that DEX treatment at both dosing regiments reversed SM-induced injury markers in rabbit corneas. However, a clear and marked dosing frequency-dependent effect was observed, with DEX treatment every 8h being more potent than 12h treatment in reversing the injury markers in our study model. The effects were most pronounced at the day 28 and day 42 timepoints post SM exposure. In conclusion, DEX administration every 8h beginning at 2h post SM exposure in the rabbit *in-vivo* ocular model was found to be more effective in reversing SM-induced corneal injuries.

Introduction

- Sulfur mustard (SM) [$C_4H_8Cl_2S$], is among the most potent alkylating chemical weapons and one of the most widely used in chemical warfare.
- SM was first used in 1917 during World War I and then in Middle-Eastern conflict (1980–1988). In Middle-Eastern conflict, there were more than 100,000 victims who suffered from several acute and delayed ocular toxicity such as photophobia, corneal ulceration, conjunctival scarring, vision loss, and recurrent corneal ulcer diseases.



1-chloro-2-(2-chloroethylsulfanyl)ethane
or Sulfur Mustard (SM)

- The mechanism of ocular injury from SM is not fully understood. There are no approved therapies, and the treatment is mostly symptom management. Despite enormous research efforts during the last 105 years, no specific sulfur mustard antidote has been found. As no therapeutic interventions or medical countermeasures are available for the treatment of eye injury from vesicant exposure, it is imperative to explore effective treatment strategies.
- Dexamethasone (DEX), an FDA approved drug, is one such treatment prospect. It has been shown to have efficacy against vesicants and can be repurposed to treat the corneal toxicity from vesicant exposure.

Methods

- New Zealand white male rabbits, weighing between 2.5–4.0 kg and a minimum of 3 months old, were obtained from Charles River Laboratories acclimatized, and housed as per approved protocol at MRIGlobal. All the experimental protocols used in this study were approved by MRIGlobal's IACUC. The animals were anesthetized at MRIGlobal's chemical surety facility, using IM administration of ketamine+xylazine+acepromazine. Once anesthetized, the rabbits were positioned on an absorptive pad, and exposed to SM vapors by securing the ocular vapor goggles around their head, for 7 min, inside the chemical hood.
- The rabbits were divided into two groups: Group I (right eye, SM exposed and left eye, control) and Group II (both eye SM exposed; right eye, DEX 12h and left eye, DEX 8h treatments). SM treatment was given at ~400 µg/L SM (390–420 µg/L; n = 5 per treatment group at each time point) for 7 min and/or 0.1% DEX formulation (Maxidex suspension, Novartis). DEX (0.1%) administration beginning at 2h post SM-exposure and then either every 8h or 12h thereafter for 28 days post SM exposure.
- Tissues were transferred to us (¾ portion snap frozen and ¼ portion formalin fixed) for further processing and analysis. Paraffin blocks were prepared followed by slide preparation with 5 µM thick sections, samples were stained with Hematoxylin and Eosin (H&E) dyes, and immunohistochemistry (IHC) was done on the tissue for protein expression analysis.

Left eye (control, unexposed) and Right eye exposed to SM (400 µg/L) for 7 min.

Clinical assessments. Animals were euthanized (at day 28, 42, and 56 post-exposure), corneas collected, ¾ portion snap frozen and ¼ portion formalin fixed for histopathological or immunohistochemical studies.

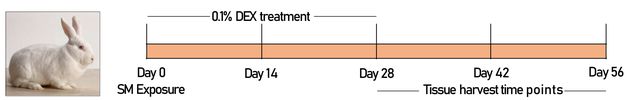


Figure 1: SM exposure and study paradigm in New Zealand white male rabbits.

Results

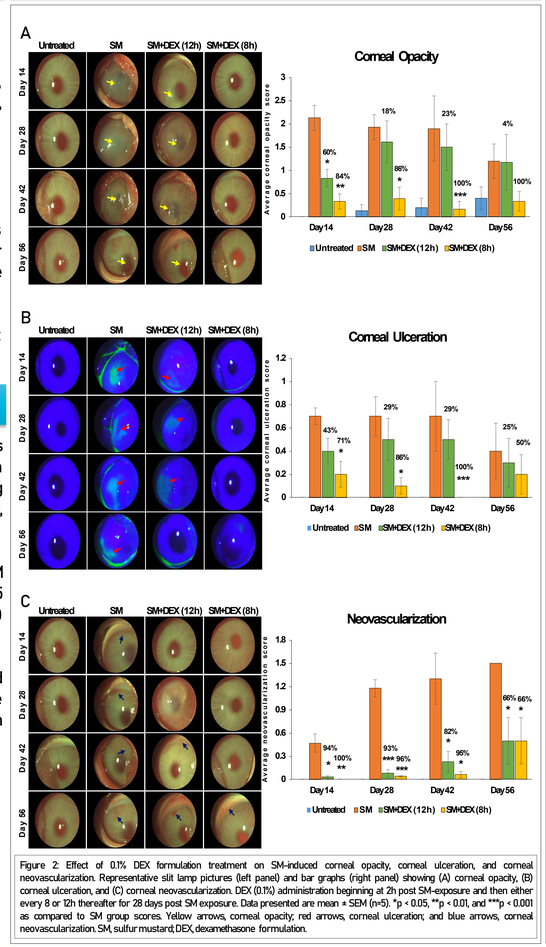


Figure 2: Effect of 0.1% DEX formulation treatment on SM-induced corneal opacity, corneal ulceration, and corneal neovascularization. Representative slit lamp pictures (left panel) and bar graphs (right panel) showing (A) corneal opacity, (B) corneal ulceration, and (C) corneal neovascularization. DEX (0.1%) administration beginning at 2h post SM-exposure and then either every 8 or 12h thereafter for 28 days post SM exposure. Data presented are mean ± SEM (n=5). *p < 0.05, **p < 0.01, and ***p < 0.001 as compared to SM group scores. Yellow arrows, corneal opacity; red arrows, corneal ulceration; and blue arrows, corneal neovascularization. SM, sulfur mustard; DEX, dexamethasone formulation.

Results

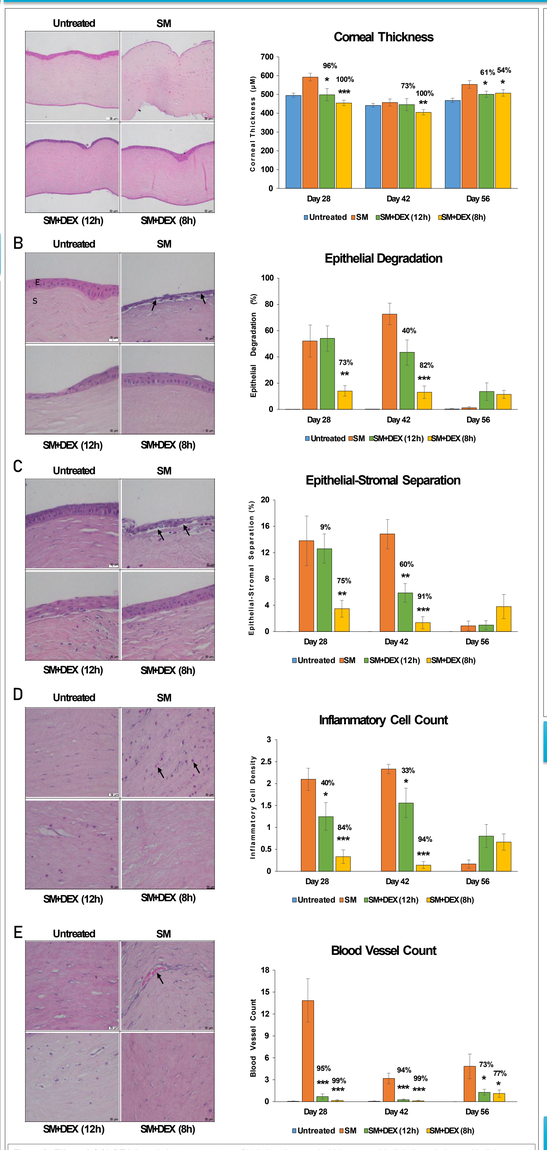


Figure 3: Effect of 0.1% DEX formulation treatment on SM-induced corneal thickness, epithelial degradation, epithelial-stromal separation, inflammatory cell count, and blood vessel count. Representative H&E images (left panel) and bar graphs (right panel) showing (A) corneal thickness, (B) epithelial degradation, (C) epithelial-stromal separation, (D) inflammatory cell count, and (E) blood vessel count. Data presented are mean ± SEM (n=5). *p < 0.05, **p < 0.01, and ***p < 0.001 as compared to SM group scores. Black arrows show the SM induced changes. SM, sulfur mustard; DEX, dexamethasone formulation; E, epithelium; S, stroma.

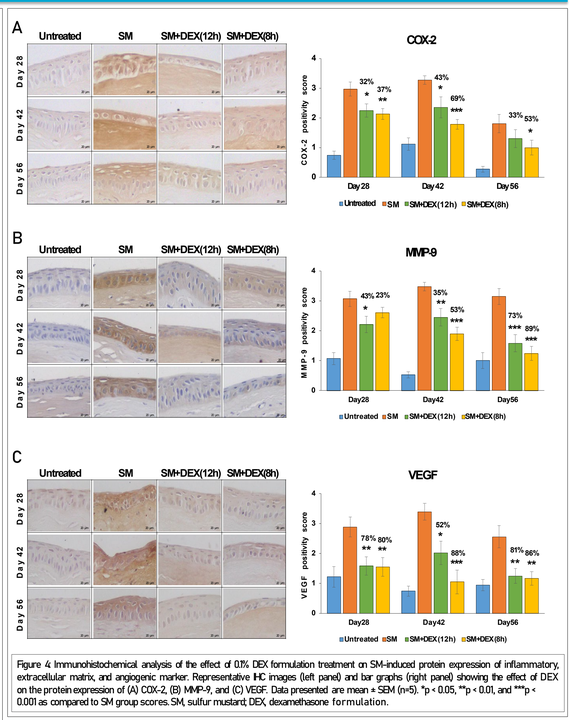


Figure 4: Immunohistochemical analysis of the effect of 0.1% DEX formulation treatment on SM-induced protein expression of inflammatory, extracellular matrix, and angiogenic marker. Representative IHC images (left panel) and bar graphs (right panel) showing the effect of DEX on the protein expression of (A) COX-2, (B) MMP-9, and (C) VEGF. Data presented are mean ± SEM (n=5). *p < 0.05, **p < 0.01, and ***p < 0.001 as compared to SM group scores. SM, sulfur mustard; DEX, dexamethasone formulation.

Summary

- The results from SM exposure in NZW rabbits here provide clinical, biological, and molecular changes associated with SM-induced acute corneal injury. The injury parameters, comprising of increase in corneal thickness, corneal opacity, epithelial degradation, epithelial-stromal separation, increase in blood vessel number, and influx of inflammatory cells, increase in COX-2, MMP-9, and VEGF levels, corroborate earlier ocular injury studies carried out with SM.
- Our results also indicate that DEX, an FDA approved drug, is effective in reversing the SM-induced injuries in rabbit eyes. Dosing frequency of 8h was more effective in reversing SM-induced corneal injuries than 12h dosing frequency; though DEX was found to be effective in reversing SM-induced injuries in both cases.
- Overall, this study establishes that DEX reverses SM induced ocular toxicity by modulating the expression of inflammatory (COX 2), extracellular matrix (MMP 9), and neovascularization (VEGF) associated molecular markers.

Acknowledgements

FUNDING: National Institutes of Health, National Eye Institute (Grant No. U01EY030405). The authors have no conflicts of interest.

The science you expect. **The people** you know.

816-753-7600 • www.MRIGlobal.org • info@MRIGlobal.org • Headquarters - 425 Dr. Martin Luther King, Jr. Blvd., Kansas City, MO 64110 | Missouri • Colorado • Maryland • Kansas