Sulfur Mustard Exposure Disrupts Transmembrane Water Channel Proteins In Rabbit Corneas *in vivo*

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Background and Rationale

- Aquaporins (AQPs) are transmembrane water channel proteins that regulate the movement of water through the plasma membrane in various tissues including cornea.
- AQPs-mediated increased water permeability facilitates the migration of cells to the site of injury during the wound healing phase.

Objective

- To investigate the effects of sulfur mustard (SM) gas exposure on AQP1, AQP3, and AQP5 transcript and protein levels in rabbit cornea in vivo.
- To examine if SM toxicity to corneal aquaporins could be mitigated by topical eye drop (TED).

Methods

- New Zealand White Rabbits exposed to SM vapor (200 mg-min/m3 for 8 mins) were divided into 3 groups: Naïve, SM, and SM+TED (2 drops/day for 4-week).
- At 2- and 4-month post SM-exposure, corneas were collected after humane euthanasia, snap-frozen, and used to generate serial tissue sections and cDNA.
- Histopathological, immunofluorescence, and qRT-PCR analyses measured changes in AQP1, AQP3, and AQP5 transcript and protein levels.
- The clinical eye tests and imaging in live rabbits were performed with slit-lamp microscope, in vivo confocal microscope, specular microscope, optical coherence tomography, fluorescein staining, Schirmer's tests, pachymetry, applanation tonometry.

Results

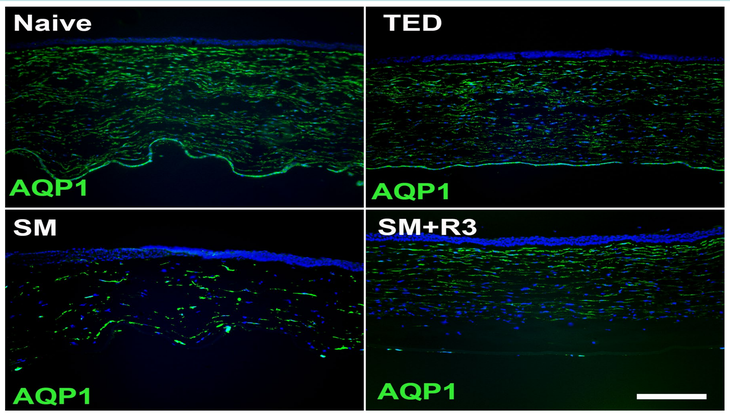

Fig. 1. Expression of Aquaporin 1 in rabbit cornea at post-SM exposure of 2 Months. SM exposed tissue shows significant reduction of AQP1, and treatment with TED shows to be somewhat effective in loss mitigation.

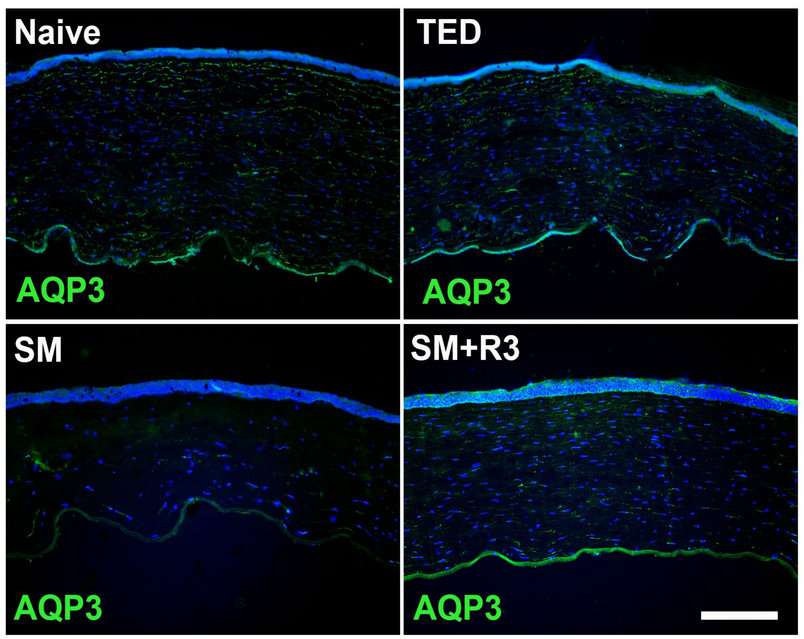

Fig. 2. Expression of Aquaporin 3 in rabbit cornea at post-SM exposure of 2 Months. SM exposed corneas show significant reduction of AQP3, however this effect is mitigated with TED.

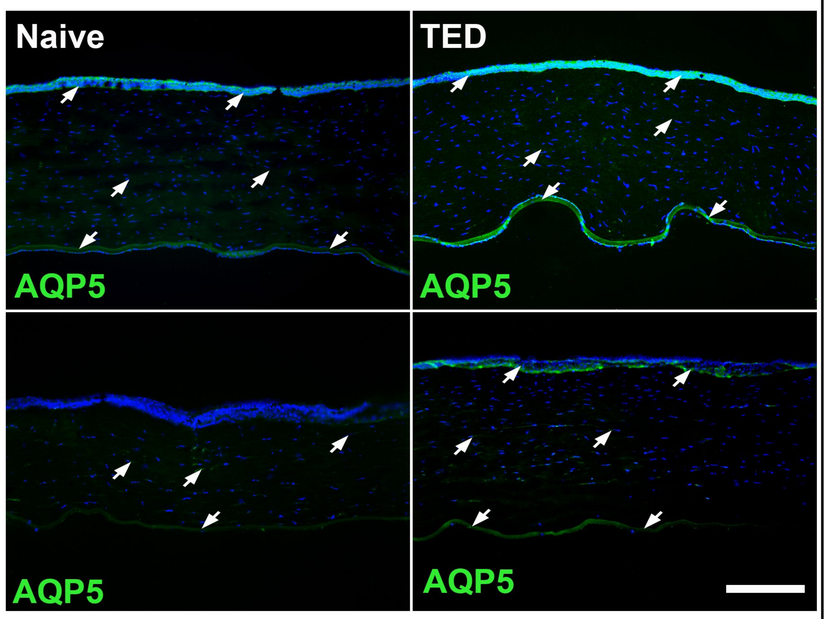

Fig. 3. Expression of Aquaporin 5 in rabbit cornea at post-SM exposure of 2 Months. While less severe than AQP1/AQP3, AQP5 expression is still significantly reduced in rabbits exposed to SM. TED treatment mitigates this reduction.

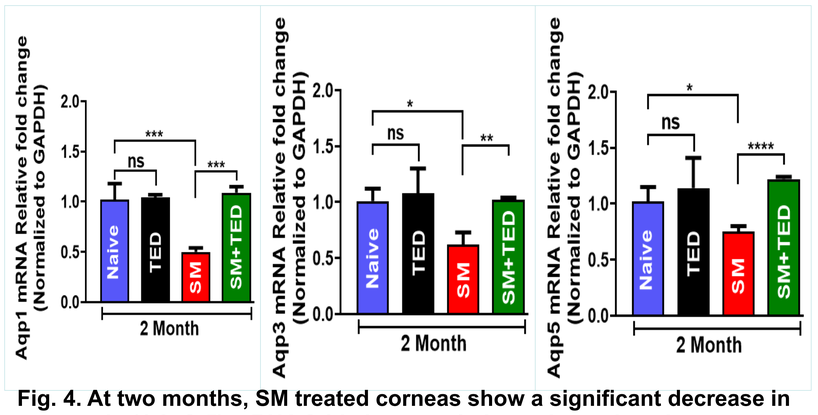

Fig. 4. At two months, SM treated corneas show a significant decrease in aquaporin (1,3, & 5) mRNA fold change relative to housekeeping genes (GAPDH). TED treated corneas show similar fold change to naïve and non-SM affected corneas.

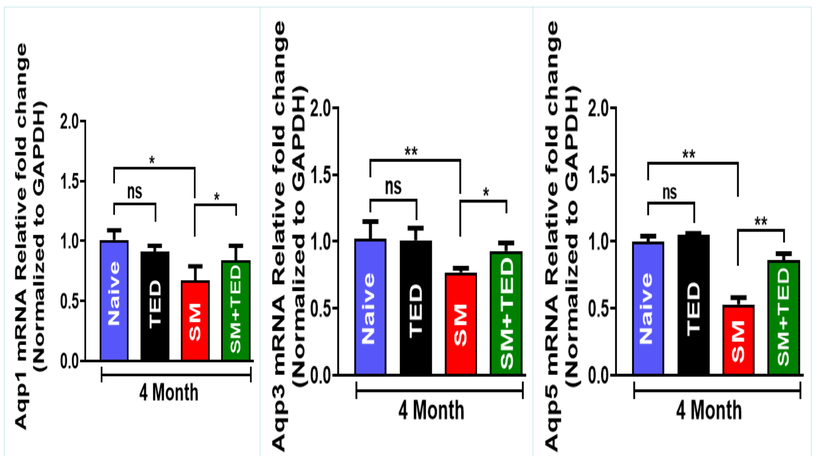

Fig. 5. At 4 months, SM treated corneas show a significant decrease in aquaporin (1,3, & 5) mRNA fold change relative to housekeeping genes (GAPDH). TED treated corneas show similar fold change to naïve and non-SM affected corneas

Conclusion

The SM-exposed rabbit corneas showed significant decrease in AQP1, AQP3, and AQP5 mRNA and protein levels compared to naïve corneas at 2- and 4-month post-SM ($p<0.001$). Furthermore, the TED treatment mitigated SM toxicity to AQPs at both tested times ($p<0.001$, $p<0.01$, $p=0.004$, $p=0.03$). These results demand in-depth investigation of aquaporin's role in MGK. Additional studies are underway.

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