Analysis of the Effectiveness of Restasis® as a Therapy for Nitrogen Mustard Induced Corneal Injuries

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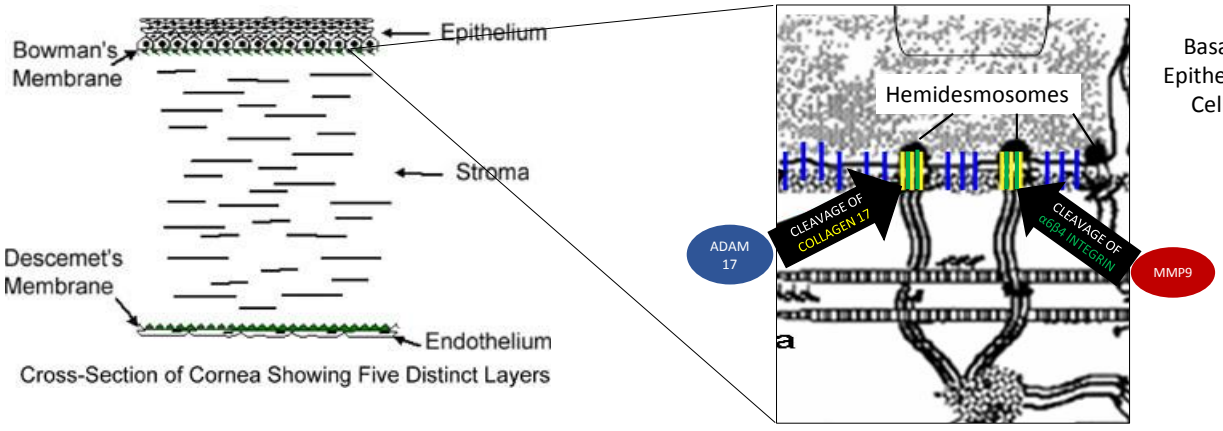
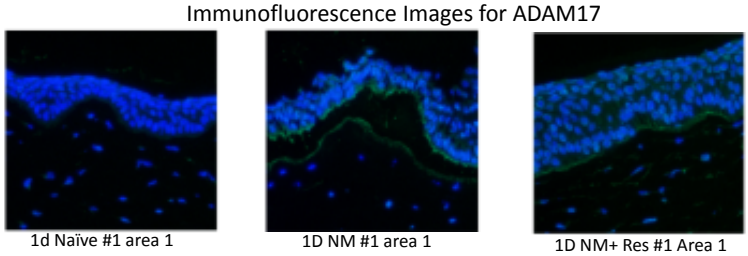
Mustard agents like nitrogen mustard(NM), are potent weapons of chemical warfare and potential weapons for terrorist attacks. Mustards are vesicants that affect the membranes of the eyes, skins, and lungs by causing cleavage of attachment molecules that hold the cell layers together, creating blisters. In the eye, micro blisters form between the corneal epithelium and stroma. Subsequent injuries range from temporary tearing and pain, to permanent blindness. The proteins cleaved in the cornea are trans-membranous anchoring components that link the basal epithelial cells to the remaining components of the hemidesmosomes, located in the basement membrane zone between the two cell layers. One of these components Collagen 17, is cleaved by enzyme ADAM 17. The other component α6β4 integrin, is cleaved by matrix metalloproteinase (MMP9). Various other proteins play key roles in the injury phase, like Cyclophilin A, and TNF-α, and the wound healing phase, like Hevin and Tenascin C. The goal of our lab was to observe the effectiveness of the FDA approved drug Restasis® in attenuating the damage caused in nitrogen mustard exposed rabbit corneas maintained in culture. Rabbit cornea cultures were divided into groups that were either unexposed or exposed to NM. Control groups included unexposed corneas that either received no further therapy (Naïve), or were treated with Restasis®. The NM corneas were also divided into groups that received therapy, or Restasis®. Corneas were then embedded in OCT agent, followed by freezing, and cryo-sectioning. Tissues were then stained by hematoxylin & eosin (H&E) to assess the injury or used to measure the expression of the various target proteins by immunofluorescence. Immunofluorescence results showed no change in target protein expression in control groups. However, the expression of target proteins peaked in corneas exposed to NM alone. This was notably diminished in corneas exposed to Restasis® after NM exposure. Also, statistical analysis of measurements of the H&E images showed an overall reduced percentage of epithelial-stromal cell layer separation in the presence of Restasis®. These results clearly establish the effectiveness of Restasis® as a therapy for NM induced corneal damage.



Immunofluorescence Images for ADAM17



1d Naïve #1 area 1 | 1D NM #1 area 1 | 1D NM+ Res #1 Area 1