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14. ABSTRACT
Eye injury from HD (sulfur mustard) exposure continues to remain a threat to soldiers in the battlefield. This study was designed to explore the effects of Pred-Forte ® (prednisolone) in treating ocular HD injury. Sixteen female New Zealand White rabbits were exposed to 0.51 mg of undiluted HD as a liquid droplet. One group (n=8) received 2 drops of Pred-Forte ® into the eye every 10 minutes for the first 30 minutes postexposure, then every 30 minutes for 2 hours, after which treatments were given three times daily (tid). The control group of animals (n=7) received 2 drops of Artificial Tears®. Eyes of rabbits were evaluated and scored weekly for 5-6 weeks, then at 12, 13 and 16 weeks. Measurements included slit lamp evaluation, corneal thickness (Pachymetry), and modified ocular severity score (MOSS). Lesions or ulcers resulting in corneal perforations developed within the first 3-4 weeks in 9 out of 15 rabbits. Treatments were discontinued at 3 weeks; however, observations continued to 16 weeks. Adverse reactions were attributed to treatment effect, inexperienced operators (HD) and/or both. The use of different applications of HD to the eye should also be considered to eliminate variability seen with the droplet method.

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ABSTRACT

Eye injury from HD (sulfur mustard) exposure continues to remain a concern and a threat to soldiers in the battlefield. Currently there are no fielded ocular therapeutics for vesicating agents such as mustard. This study was designed to explore the effects of the commonly prescribed FDA approved ocular drug Pred-Forte® (prednisolone) in treating ocular HD injury. Sixteen female New Zealand White rabbits were divided into 2 groups (n=8). Rabbits were exposed to 0.4 µl (0.51 mg) of undiluted HD as a liquid droplet for 5 min. One group (n=8) received 2 drops of Pred-Forte® into the eye every 10 minutes for the first 30 minutes, beginning at 10 minutes postexposure. Treatment continued in this group every 30 minutes for 2 hours postexposure (total treatments at 2 hours = 6), after which treatments were given three times daily (tid). The control group of animals (n=7) followed the same schedule receiving 2 drops of Artificial Tears® (control) as the treatment. Treatments (tid) for all groups were to continue for 6 weeks. Eyes of rabbits were evaluated and scored weekly for 5-6 weeks, then at 12, 13 and 16 weeks. Measurements included slit lamp evaluation, corneal thickness (Pachymetry), and modified ocular severity score (MOSS). Lesions or ulcers resulting in corneal perforations developed within the first 3-4 weeks in 9 out of 15 rabbits. Treatments were discontinued at 3 weeks; however, observations continued to 16 weeks on the remaining 6 rabbits. Adverse reactions were attributed to treatment effect (prednisolone), inexperienced operators (HD) or a combination of both. The use of different applications of HD to the eye should also be considered to eliminate variability seen with the droplet method.

PRED FORTE® (prednisolone acetate) 1% sterile ophthalmic solution manufactured by Allergan America. Artificial Tears® (polyvinyl alcohol 1.4%) distributed by Phoenix Pharmaceutical, Inc.

INTRODUCTION

The use of sulfur mustard (HD) in recent military conflicts such as the Iran-Iraq war and the continuing threat of its use in future conflicts have accelerated research efforts to develop effective therapy for prevention and treatment of HD-induced vesicant injury (1-2). HD produces severe ocular impairment as well as serious cutaneous and respiratory damage (3-6). Previous ocular experiments were performed on a rabbit model documented in volume XIV Medical Aspects of Gas Warfare in 1926 (7). The research performed was a natural history study detailing the effects of liquid sulfur mustard exposure in a rabbit eye model. This research exquisitely addressed the devastating nature of sulfur mustard ocular damage from 5 minutes to 7 weeks postexposure. Photo documentation of gross pathology and microscopic pathology was performed and recorded. No prophylactic or postexposure treatment modalities except for eye flushing with water were performed.

The full mechanism of action of HD on the eye (and skin) is still under investigation, as is the development of pretreatment or treatment compounds that can decrease the effects of ocular exposures of this compound in humans. There are no fielded therapeutics for vesicating agents at this time. In addition, HD and chlorovinyldichloroarsine (Lewisite, L, the other most commonly employed vesicating agent) exposures to the ocular structures for as short as 5 minutes produce severe biochemical changes that are manifested after an initial latent period (6).

The eyes are the most sensitive organ to the effects of sulfur mustard exposure. In response to mustard exposure an acute reaction typically results in photophobia, conjunctivitis, and corneal inflammation. The high turnover rate of corneal epithelial tissue makes this later structure particularly sensitive to mustard. The immediate intramolecular cyclization and resulting cyclic intermediates of mustard in the eye tissue (or skin) result in chromosomal aberrations, and inhibition of DNA and RNA. This ultimately leads to corneal damage and if severe enough can result in corneal denudation, erosions and potential perforations due to loss of structural integrity (1, 8, 9). The later stages of mustard injury are characterized by an increase in inflammatory infiltrates of connective tissue and epithelial regeneration of the cornea. If the stromal region is damaged regeneration is incomplete and can result in recurrent erosions, ulcers and vascularization (6-9). Inflammatory cellular infiltrates were reported as common responses in the skin and corneal stromal regions of the eye (1, 10).

Ocular injuries due to mustard exposure continue to present a challenge to the clinician. Although eye structures are more sensitive and can be more seriously affected by HD, surrounding skin damage also needs to be considered. Most conventional treatments against mustard ocular injuries are symptomatic (4). These include irrigation followed by topical antibiotics for secondary bacterial infections and a mydriatic agent to prevent synechiae. Recently, treatments for mustard ocular injury have focused on interdicting at the inflammatory phase of the injury, which may have a more important role in HD injury than previously believed. Mustard has been found to cause the release of many primary inflammatory mediators (e.g. TNF-α, IL-1) in skin (11) and is likely to contribute to the same release of mediators in the eye. Other potential modes of ocular treatments focus on PARP inhibitors, scavengers, or protease inhibitors. Many of these treatments have shown promising results in HD-induced skin injury (12).

Steroid treatments are common ophthalmic preparations used frequently as anti-inflammatory treatment agents for ocular injuries, iritis, superficial punctate keratitis, and allergic conjunctivitis (13, 14). They are also used adjunctly for many corneal injuries, ranging from chemical, radiation or thermal burns, to help reduce inflammation and edema. Prolonged use of ocular steroid treatment, however, may have several detrimental effects. For example, corticosteroids suppress host

responses and can consequently increase the risk of secondary ocular infections particularly in acute infections (13, 14).

Anti-inflammatory agents (both steroidal and nonsteroidal anti-inflammatory drugs) and other potential classes of treatments have been shown to be effective in inhibiting corneal neovascularization in a wide spectrum of clinical and experimental situations (15-17). Kadar and colleagues have documented inhibition or delay of corneal neovascularization in HD vapor-exposed rabbit corneas treated with dexamethasone ophthalmic drops three times a day for two weeks duration (17). Rat studies have indicated that in animals dosed with three times the LD50 dose of HD and injected with various drugs 30 minutes later, the best protective effect (decreased lethality, fewer pathologic organ changes, less loss of body weight) was obtained with a combination of sodium thiosulfate (HD scavenger), Vitamin E (anti-oxidant and free radical scavenger), and dexamethasone (corticosteroid with anti-inflammatory effects) (18). It therefore makes sense to utilize some of these compounds in ocular preparations to observe possible beneficial effects on the ocular tissues exposed to HD. A combination steroid/antibiotic would be best studied first since these agents are already commercially available and in widespread ophthalmic use.

Corticosteroids are also known to delay the rate of corneal-epithelial regeneration (19). In diseases or processes such as mustard exposure that already cause thinning of the cornea perforations can be a potential risk. Steriods can greatly potentiate the effects of collagenase activity, resulting in severe destruction (melting) of the cornea (19). Amir et al. (20) reported a slight delay in corneal erosion healing with steroid treatment (2 weeks) but did not report any ruptures in their dexamethaxone studies. Short-term, early use of steroids and/or combination treatments (e.g., steroid and antibiotic), however, have shown favorable results by interacting at the inflammatory stages. Human keratinocytes exposed to HD have been found to exhibit the capability to release mediators such as IL-1, IL-6, IL-8, and TNF-α (21). These cytokines are normally within the cell but are released when cells are damaged and contribute to the inflammatory response.

The topical application of steroid/antibiotic combination ophthalmic drops has recently been studied during the development of the rabbit eye model here at this institute (22, 23). Unfortunately, only a very modest and short-lived beneficial effect was seen in those animals treated with topical ophthalmic drops (triamcinolone/cefazolin). As concluded by these investigators this was most likely secondary to low drug concentrations in the target tissues. (22). In addition, more work is needed on the role and potential use of steroids and their effect as treatments for mustard-exposed ocular injury.

This study was designed to address the effects of a topical ophthalmic steroid preparation as a potential treatment for mustard-induced ocular injury. Steriods are often used in combination but the effects of just the steroids alone have only been studied in a variety of ocular conditions and not as an antivesicant (HD) treatment. The goal of this study was to determine the effects of prednisolone acetate (Pred-Forte®), a commonly available ophthalmic steroid preparation, on the healing of HD-induced ocular injury.

MATERIAL AND METHODS

Sixteen female New Zealand White rabbits weighing 2.5-3.0 Kg were used in this experiment. Animals were maintained under an AAALAC accredited animal care and use program. They were quarantined and observed for evidence of disease for five days prior to issue and were housed singly in a stainless steel cage (52.8 cm L x 52.8 cm W x 37.4 cm H). Animals received a complete cage change weekly or were changed as needed to maintain a clean environment. During quarantine and continuing throughout the study rabbits were handled daily and groomed particularly around the eyes to acclimate them to the procedures. They were also acclimated to environmental enrichment that included daily runs in a small 4' x 4' pen. The pen had a variety of toys that included PVC tubing, dumbbell toys, and shaker toys with bells.

Rabbits were provided a certified commercial rabbit ration (PMI, St. Louis, MO; 125 g/day), tap water ad libitum and regular fruit and vegetable treats. Animal holding rooms were maintained at 20°C ± 2° with 45.0% ± 10% relative humidity with at least 10 complete air changes per hour of 100% conditioned fresh air. A 12-hour light/dark, full spectrum lighting cycle with no twilight was maintained in all animal holding areas. While in the fume hood after exposure (24-hour duration) rabbits were housed in a Kennel Cab II® (A.J. Buck and Sons, Owings Mills, MD; 12" H x 22" L x 14" W). This carrier was chosen to fit within the confines of the hood and has a raised floor to keep the animal clean.

One week prior to study rabbits were lightly sedated with an i.m. injection of 7 mg/Kg Ketamine HCl in combination with 3.5 mg/Kg Xylazine for minor procedures of tattooing, nail clipping, and a screening ophthalmologic exam for any preexisting lesions and/or other abnormalities. Hair was clipped around the eyes, ears and back of the animal in preparation for HD exposure and osmotic pump implantation. Rabbits were tattooed using an AIMS machine (Animal Identification and Marking Systems; AIMS, Budd Lake, NJ) for placing permanent identification numbers inside the right ear.

On the day of the study rabbits were sedated with an i.m. injection of 15 mg/kg Ketamine HCl in combination with 7 mg/Kg Xylazine and transported to the agent dosing area for HD exposure and surgical placement of osmotic pumps (buprenorphine HCl). Pachymetry measurements for corneal thickness were taken on both eyes in triplicate followed by slit lamp (Haag-Streit Services, Mason OH) and whole eye examination by a board certified ophthalmologist. A modified ocular severity score (MOSS) was recorded. Pictures were taken and recorded of the eyes using an Image-Pro plus program software package (Media Cybernetics®, Silver Spring, MD).

Following the ophthalmologic examination, drug delivery pumps (Alzet osmotic pump, model 2ML1-10, 10µl/hr, 7 days) containing buprenorphine hydrochloride (0.3 mg/ml) were aseptically implanted in the experimental animals for pain alleviation. Pumps were implanted mid scapula and the incision was closed with surgical staples. Prior to implantation, pumps were weighed before and after filling to give the net weight of the solution loaded. Once implanted, the pumps delivered continuous and constant infusion of pain control medication for up to a week, after which the pumps were removed aseptically.

Exposure to HD liquid was accomplished using the methods described in SOP 91-067-DB-01, "Surety Procedures for Cutaneous Applications of Sulfur Mustard (HD) on the Skin of Laboratory Animals." A small liquid droplet of neat HD (0.4 µl, 0.51 mg) was placed into the right eye (held open with an eye speculum) of the rabbit using a Gilson Pipetman P-2 pipetter, and agent was allowed to remain on the eye for 5 minutes after which the eyelid was manually blinked several times. There were no special decontamination procedures done on the eye other than the treatments and light wiping off of excess tears from the eyes as needed using gauze or surgical wicks on the day of exposure. Animals remained in the fume hood for 24 hours after exposure as determined by off-

gassing evaluations. While in these carriers the animals were fed, watered, and treated by the project technicians. After 24 hours rabbits were returned to the colony room and tid. treatments and evaluations continued.

Rabbits were randomly assigned to one of two treatments (Pred-Forte® - prednisolone acetate or artificial tears solution). Following exposure one treatment group of rabbits (n=8, even number rabbits) received 2 drops of Pred-Forte® into the eye every 10 minutes for the first 30 minutes, beginning at 10 minutes postexposure. Treatment continued in this group every 30 minutes for 2 hours postexposure (total treatments at 2 hours post = 6), at which time they were started on a tid schedule (0800, 1200, 1700). The other treatment group of animals (n=7, odd number rabbits; one animal died due to anesthesia, see Results section) followed the same schedule with 2 drops of Artificial Tears® (control) as the treatment. For ease of resources and personnel rabbits 1-8 were exposed one week and rabbits 10-16 were exposed the following week.

Treatments (tid) for all groups continued to the end of the study, which was intended to last 6 weeks. In addition to the Pred-Forte® or artificial tears treatment, rabbits were supportively treated around the eyes with warm water to break up adhesions or discharges that commonly sealed the eyes closed. Eyes of rabbits were evaluated and scored weekly for 5-6 weeks, then at 12, 13 and 16 weeks. During evaluations rabbits were placed in Lomir "Bunny Snuggle" restrainers for approximately 10-15 minutes. One to 2 drops of tetracaine ophthalmic solution was placed on the corneas of non-anesthetized rabbits in order to perform ophthalmic examinations. On rare instances only was a rabbit sedated for evaluation for a more thorough exam. In these cases an i.m. injection of 7 mg/Kg Ketamine HCl in combination with 3.5 mg/Kg Xylazine was given for anesthesia.

The degree of injury and rate of healing were evaluated using several instrumental techniques. Measurements included slit lamp evaluation, corneal thickness (Pachymetry), and a modified ocular severity score (MOSS). An ultrasonic pachymeter (PR, DGH200, DGH Technology, Inc. Easton, PA) was used to measure corneal thickness. Measurements were made in a standardized manner at the area of injury. Using a subjective MOSS grading scheme (Table 1) and a slit lamp examination, documented with photography, scoring was used to evaluate and quantify corneal stromal injury and scarring, neovascularization, chemosis and eyelid damage (notching).

At the conclusion of the study (16 weeks) and after a final ophthalmology exam rabbits were euthanized. Euthanasia was accomplished by first administering an i.m. injection of Ketamine:Xylazine (15.0 mg/Kg:7.0 mg/Kg), then while the animal was in a surgical plane of anesthesia, an intracardiac injection of Fatal Plus (65 mg/kg: 390mg/ml pentobarbital) was administered. Gross examinations of abnormalities of the eyes and their adnexa as well as microscopic examination of all ocular structures were conducted. Sections were examined through the bulbar conjunctiva, the lids and adnexa, and a horizontal, vertical, or oblique axis P-O (pupil-optic nerve) section through the entire globe specifically including the cornea. Lesions in adnexal structures were rated for severity (0 = no lesion, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe) considering mucosal damage, mucosal inflammation, cleft or pustule formation, submucosal damage and submucosal inflammation. Corneal lesions were similarly rated for severity (1-4), considering epithelial damage, stromal damage, stromal edema, stromal vascularization and inflammation. Diagnostic necropsies were performed on animals that had died during the course of the experiment.

RESULTS

Sixteen rabbits were assigned to the study; however, one rabbit died due to anesthesia-related complications prior to exposure as documented at necropsy. Treatments were then allocated as 8 rabbits receiving Pred-Forte® ("Test") and 7 rabbits receiving artificial tears ("Control"). All rabbits except one (#4, a Pred-Forte®-treated rabbit) developed blephorospasm, photophobia, conjunctivitis and corneal edema within 48 hours of exposure. These same rabbits also developed mild to moderate skin edema and erythema around the adnexa of the eye and a mucopurulent discharge starting at 24-48 hours postexposure. Rabbit #4 showed only mild blephorospasm for the first 24 hours and a mild serous discharge that resolved within 48 hours. This rabbit then remained clinically normal the remainder of the study, and for this report will only be referenced to in summaries.

Table 2 summarizes the overall results for the 16 rabbits and the final clinical observations for each rabbit at euthanasia. By week one, as determined by MOSS scoring, 10 of the 14 rabbits had developed eyelid notching (7 treated, 3 controls), and 7 of the 14 demonstrated chemosis in the exposed eye (5 treated, 2 controls). In addition, corneal stromal injury was evident in all rabbits except 1 (rabbit #13) by the second week after exposure. Rabbit #13 (control-treated) developed only a mild hazy cornea and conjunctivitis throughout the course of the 4 weeks. Two rabbits (Pred-Forte®-treated-rabbits) required euthanasia within the first week, and 4 rabbits (3 Pred-Forte®-treated rabbits, 1 control-treated rabbit) required euthanasia within the second week for injuries developing in the cornea that resulted in corneal perforations. In the 8 remaining rabbits severity of injuries continued to develop (except #13) to week 4 postexposure, resulting in 3 more euthanasias due to perforated corneas (1 Pred-Forte®-treated rabbit in week 3, 2 control rabbits, one at week 3, one at week 4). Because of the increase in corneal perforations and the severity of clinical signs noted by the third week and the decreasing number of rabbits in both groups, it was decided to discontinue treatments at the third week (other than the supportive cleaning around the eyes). Table 3 depicts the results of the MOSS scoring.

Pachymetry readings showed an increase in values demonstrating an increase in thickness particularly in the control-treated eyes within the first 4 weeks. Treated rabbits also showed an increase but not as high as control-treated rabbits. By 6 weeks, measurements had decreased but still not to levels seen when compared with the unexposed left eye. Results of pachymetry are illustrated in Figure 1. By week 4 neovascularization was beginning to develop mildly in 4 of the remaining 5 rabbits exposed (excluding rabbit #4, 2 treated, 2 controls), and by 6 weeks these 5 rabbits exposed had developed at least one new neovascularization site. This clinical sign continued to develop in the 5 rabbits until euthanasia at 16 weeks.

There was a sooner tendency and a greater chance for corneal ulceration in the Pred-Forte® treated rabbits than in control-treated animals; however, significance could not be reliably determined due to the decreased numbers of animals (i.e., no significance at $p < 0.05$). Overall Pred-Forte®-treated rabbits had more perforations than control-treated rabbits by 4 weeks postexposure (6 of 7 Pred-Forte®-treated vs 3 of 7 control treated rabbits). It was also noted that 6 of 7 animals exposed the first week (Rabbits 1-8) developed perforated corneas, while the corneas of only 3 of 7 animals exposed the second week (Rabbits 10-11) became perforated. In this study there were 3 different operators, of which 2 were inexperienced in the actual application of HD to the eye. The more experienced operator had 3 of 5 animals with perforated corneas, while the two inexperienced operators had 4 of 6 animals and 2 of 4 animals respectively developed perforations. Results are reported as observations only since no significance was noted in any of the parameters measured.

Pathology results noted severe corneal epithelial ulceration, necrosis, and edema as well as moderate to severe corneal and conjunctival inflammation in all rabbits euthanized by 4 weeks. The

remaining 5 rabbits that were euthanized at 16 weeks showed varying degrees of corneal ulceration, necrosis, and inflammation. Neovascularization tended to be a later event, first appearing at 3-4 weeks. In those rabbits euthanized early, severe damage to the corneal endothelium was also noted at the time of euthanasia. Corneal epithelial necrosis was noted only in one out of the 5 rabbits at 16 weeks. Results of the pathology scoring are tabulated in Appendix 1-3.

DISCUSSION

Kadar and colleagues have documented beneficial effects using only dexamethasone in HD vapor-exposed rabbit corneas (17, 20). Kadar et al. (17) demonstrated that tid treatments of dexamethasone for 2 weeks delayed (but did not prevent) corneal neovascularization. In a follow up study, Amir et al. (20) hypothesized that anti-inflammatory treatments would have beneficial effects by preventing some aspect of the primary development of HD-induced lesions (e.g., release of inflammatory mediators). Their results showed that early application of dexamethasone (1 hour after exposure then tid for 2 weeks) reduced the acute inflammatory response as measured by biochemical markers (e.g., PGE, protein) and clinical observations.

In our study the longer duration (3 weeks) of prednisolone treatment may have directly or indirectly resulted in the corneal perforations. Although it could not be definitively determined whether prednisolone was the cause for the perforations due to the low numbers of animals, it seemed likely that the steriod did have a contributing role particularly in corneas that were thinning and attempting to regenerate. Prednisolone-treated eyes tended to show lower pachymetry readings (representing thinner cornea) than control-treated animals; however, no significance was determined ($p < 0.05$) due to the lack of sufficient numbers of animals. This may have indicated a delay in healing and therefore a defect in the cornea possibly arresting or inhibiting any further healing. Conceivably continued steroid use alone worsened an already weakened corneal structure due to increase collagenase activity and consequently resulted in ruptures. It is interesting to note that the package insert listing indications and contraindications for Pred-Forte® specifically cite under warnings that "corticosteroids are not effective in mustard gas keratitis (and Sjorgren's) keratoconjunctivitis"; however, no reference was given in the package insert for this claim. The differences seen in this study from the studies by Kadar et al. and Amir et al. (17, 20) could be in the duration of treatment (2 weeks for dexamethasone vs 3 weeks for prednisolone) or in the method of mustard exposure (vapor vs droplet).

The model developed in previous investigations and used in this study was designed to address ocular injury structures. The droplet application of HD consistently results in an ocular and surrounding skin reaction. The Gilson Pipetman P-2 pipetter was used in this study as well as in past studies for applying a liquid droplet of neat HD. The dose of HD used was also similar to that used in previous studies here at the institute (22-23). Although the droplet method is the quickest and simplest method it may not be the most ideal. Depending on the operator the delivery of agent can vary.

Several problems occurred during the study that may have been related to the development of lesions or ulcers and the resulting perforations that occurred within the first 3-4 weeks. This study was initiated when all former technicians (except one) and investigators were no longer at the institute. Although one technician was available and practice sessions were conducted with former investigators, the inexperience of the team may have had a negative impact on the results. For an inexperienced operator it is often difficult to visualize the small amount of agent and then apply it uniformly to an area of the cornea with the pipette. The small droplet applied is concentrated on only one area of the cornea and does not spread evenly over the cornea. In addition, pipette

techniques may have resulted in inadvertent trauma to the cornea, varying amounts of mustard applied, and differences in the location of the mustard droplet on the cornea that may have complicated measurements and analysis. All these factors could easily have resulted in a minor corneal abrasion in some animals that allowed the concentrated mustard to cause more severe damage to that area of the eye, resulting in perforations. Techniques may also explain the lack of response in one rabbit (rabbit #4, and possibly #13) to typical mustard exposure clinical signs.

In the studies conducted by Kadar et al. (8, 17) and Amir et al. (20) mustard was applied via goggles as a vapor to the entire eye and adnexa. This model allows for a more uniform exposure of mustard to an entire surface of the eye and a portion of the skin surrounding the eye, which more closely mimics a real life situation. Although this model mimics reported HD exposure in humans, experimental treatments can be confounded by the surrounding skin responses to HD and the potential for secondary bacterial infections from surrounding dermal tissue. Schultz et al. (24) developed a rabbit model to address treatments of alkali-injured corneas. In their studies they used a corneal block and a vacuum trephine apparatus to apply a sodium hydroxide alkali burn to the center of the cornea only. Their results allowed for a true ocular injury and uncomplicated analysis. Although this model would have the advantage of simplifying the analysis of a pure ophthalmic therapy for HD-induced ocular injury, it would not be able to evaluate a more realistic scenario of skin and ocular involvement after HD exposure.

All rabbits (except #4) developed moderate to severe eyelid swelling and conjunctivitis that would often result in adhesions of the eyelids, complicating treatments. In many cases the skin reactions around the eye, much like that commonly seen in skin after mustard exposure, results in a secondary bacterial infection that can complicate treatments (6). In this study this was routinely treated supportively with warm water rinses around the skin of the eyes prior to delivery of the treatments. This is a common complication of the application of the mustard via droplet method and, despite early supportive care in this manner, was a consistent confounding problem. Possible solutions to this complication could be more aggressively pursued in future studies. These include the use of a vasoline or similar base type ointment around the skin or more frequent cleanings. Alternatively a vacuum delivery system could be utilized as in Schultz et al. (24).

Damage to tissues or foreign insults often allow opportunistic bacterial organisms to cause secondary infections that release inflammatory mediators and cascade events that change the tissue environment. These changes can lead to circulatory alterations, necrosis of tissue and breakdown of cellular structures. For these reasons it is traditionally recommended to treat mustard gas keratitis with antibiotic drops to prevent secondary infections (1). The administration of other symptomatic treatments such as mydriatics and systemic analgesics is also indicated for HD-induced injuries.

The administration of prednisone in combination with antibiotics has recently shown to be successful in treating mustard exposure. In an early study, Babin et al. (22) reported extremely effective results using either one subtenon injection of triamcinolone/cefazolin 10 minutes after HD or a subtenon injection of triamcinolone/cefazolin 10 minutes after HD followed by a second or third injection at 7 or 14 days after HD exposure. Later Babin et al. (23) reported successful response with approved ocular treatments of prednisone acetate given early (10-min intervals to 30 min then 60, 90, and 120 min) after HD exposure followed by subtenon injection of triamcinolone/cefazolin. The rationale for this treatment regimen is to control the secondary bacterial infection (antibiotic) while also blocking the cascade reactions (steroid) and subsequent damage to tissue that occur with inflammatory mediators.

Finally as an additional note, there were several other complications that developed throughout the course of this study that could have contributed to the complications noted. In addition to the lack of experience of HD operators, pachymetry readings tended to vary greatly even within a given animal. Depending on the operator and the amount of pressure applied to the probe, measurements

can vary. Early in the study (up to 4 weeks) the ophthalmologist was the only individual who scored (MOSS) and measured corneal thickness via pachymeter. Unfortunately, this individual was not able to complete the study, requiring a different individual to finish scoring and pachymetry measurements to 16 weeks. This may have adversely affected later evaluations and analysis.

In conclusion, based on current literature it would seem that corticosteroids do play an important role in therapy of HD-induced ocular injury. However as potentially demonstrated in this study their use must be carefully considered. The consensus seems to be that the judicious use of steroids in HD exposure is warranted for at least the short term and may be best used as an adjunct to another treatment regimen (e.g., antibiotics). This study did demonstrate the need for an effective model that can more accurately assess treatments and allows for a discussion of two other models for applying HD as a vapor (vapor cap/goggles vs vacuum trephine apparatus). Depending on the need one of these two models can be developed in-house to address a more complete analysis and treatment (vapor cap method) or corneal injury alone therapy (vacuum trephine). The complications of the droplet method may not warrant this model as the most ideal for future studies. Future studies should consider developing the rabbit eye model further and continue using combination treatments aimed at maximizing healing.

TABLE 1. MOSS (Modified Ocular Severity Score) definitions

Parameter Evaluated	Parameter Score	Evaluation
Corneal Stromal Injury	0	No haze to cornea
	1	Minimal haze to cornea
	3	Moderate haze to cornea
	4	Extensive haze to cornea
NV Classification	0	No NV present
	2	One individual (twig) NV site present
	4	Two or more individual (twigs) NV sites present
	6	Diffuse NV (C or fan-shaped)
Eyelid Notching Present	1	Yes
	0	No
Chemosis Present	1	Yes
	0	No
Total MOSS Score	0 to 12	

NV = neovascularization

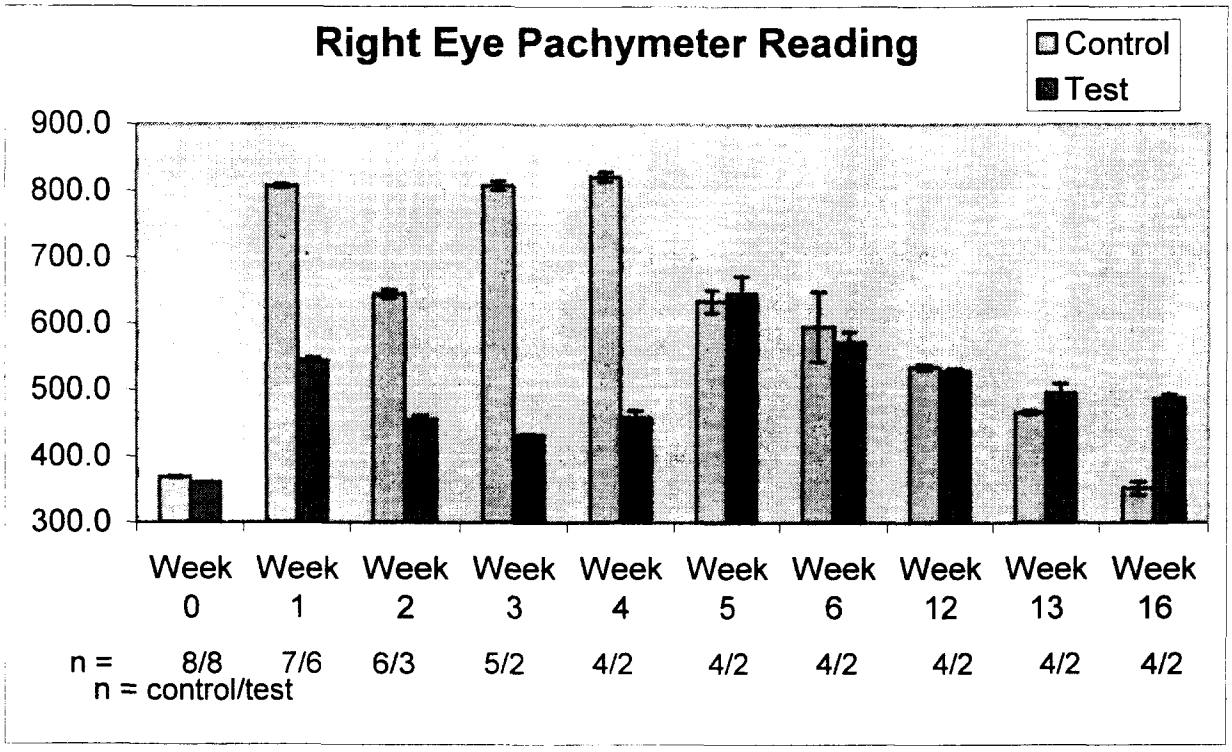
TABLE 2: Summary of rabbit ocular study

ID	date HD exposed	Operator	treatment group	Comments at euthanasia	Euth date
1	4/29/02	Tech 1	Control-art tears	Perforated cornea week 3	5/21/02
2	4/29/02	Tech 1	Pred-Forte®	Perforated cornea week 3	5/21/02
3	4/29/02	Tech 2	Control-art tears	Perforated cornea week 4	5/28/02
4	4/29/02	Tech 2	Pred-Forte®	Suspect no HD exposure-normal	8/19/02
5	4/29/02	Tech 2	Control-art tears	Moderate corneal damage and neovascularization	8/19/02
6	4/29/02	Tech 2	Pred-Forte®	Perforated cornea week 2	5/14/02
7	4/29/02	Tech 2	Control-art tears	Perforated cornea week 2	5/14/02
8	4/29/02	Tech 2	Pred-Forte®	Perforated cornea week 2	5/14/02
9	Not exposed	0	None	Died on exposure day-anesthesia related	5/6/02
10	5/6/02	Tech 1	Pred-Forte®	Mod-severe corneal damage	8/26/02
11	5/6/02	Tech 1	Control-art tears	Mod-severe corneal damage	8/26/02
12	5/6/02	Tech 1	Pred-Forte ®	Perforated cornea week 2	5/21/02
13	5/6/02	Tech 3	Control-art tears	Mild corneal damage	8/26/02
14	5/6/02	Tech 3	Pred-Forte®	Perforated cornea week 1	5/15/02
15	5/6/02	Tech 3	Control-art tears	Mild corneal damage	8/26/02
16	5/6/02	Tech 3	Pred-Forte®	Perforated cornea week 1	5/15/02

TABLE 3 : MOSS score results from control-treated rabbits (artificial tears) and test (Pred-Forte®) treated rabbits exposed to HD in the right eye.

	Control			Test		
MOSS	**Avg**	**sem**	**n**	**Avg**	**sem**	**n**
Week 0	0.0	0.0	8	0.0	0.0	8
Week 1	3.0	1.04	7	5.0	1.29	6
Week 2	6.1	2.04	6	2.6	2.60	3
Week 3	7.8	2.40	5	13.0		1
Week 4	3.0	2.00	4	44.0		1
Week 5	8.3	1.45	4	14.0		1
Week 6	12.0		4	0.0		1
Week 12	5.3	1.93	4	3.5		1
Week 13	4.8	1.03	4	4.5		1
Week 16	4.3	1.93	4	4.0		1

FIGURE 1: Pachymetry readings for control (artificial tears) and test (Pred-Forte®) treated rabbits exposed to HD in the right eye (mean ± sem) .



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APPENDICES

Appendix 1. Pathology results (scoring) for left and right eye rabbit (RB) numbers 4, 5, 10 (treated), 11, 13, 15 (control).

						0= No lesion; 1= Minimal (1-10%); 2= Mild (11-25%); 3= Moderate (26-45%); 4= Severe (>45%) NE=not examined NP=not present							
Animal number	Eye R(right) or L(left)	cornea epithelium ulceration	cornea epithelium necrosis	cornea epithelium attenuation	cornea stromal necrosis and loss	cornea stromal edema	cornea stromal neovasc	cornea stromal inflammation	cornea stromal deformity	Cornea endothelium	conjunctiva necrosis/ulceration	conjunctiva inflammation	Comments
RB4	L	0	0	0	0	0	0	0	0	0	0	1C	
RB4	R	1	0	2	1	2	2	2A	2	1	0	2C	Mild corneal keratinization and goblet cell metaplasia.
RB5	L	0	0	0	0	0	0	0	0	0	0	2C	
RB5	R	3	0	3	2	3	3	3	3	0	0	0	
RB10	L	0	0	0	0	0	0	0	0	0	0	0	
RB10	R	4	4	3	3	3	4	4CA	4	3	2	3CA	
RB11	L	0	0	0	0	0	0	0	0	0	0	0	
RB11	R	4	0	4	4	4	4	3A	4	2	0	1C	
RB13	L	0	0	0	0	0	0	0	0	0	0	0	
RB13	R	0	0	0	0	0	1	1C	0	0	0	0	
RB15	L	0	0	0	0	0	0	0	0	0	0	0	
RB15	R	0	0	1	0	0	1	0	0	0	NP	NP	

Appendix 2. Pathology results (scoring) for left and right eye rabbit (RB) numbers 1, 7 (control), 2, 6, 8, 14, 16 (treated).

Animal number	Eye R(right) or L(left)	cornea epithelium ulceration	cornea epithelium necrosis	cornea epithelium attenuation	cornea stromal necrosis and loss	cornea stromal edema	cornea stromal neovasc	cornea stromal inflammation	cornea stromal deformity	Cornea endothelium	conjunctiva necrosis/ ulceration	conjunctiva inflammation	Comments
RB06	L	0	0	0	0	0	0	0	0	0	0	0	
RB06	R	4	4	0	4	4	0	4A	0	0	0	4A	Great corneal pustule; diffuse congestion of coroid.
RB07	L	0	0	0	0	0	0	0	0	0	0	0	
RB07	R	4	4	4	4	4	4	4A	0	4	3	3CA	Conjunctiva - goblet cell loss
RB08	L	0	0	0	0	0	0	0	0	0	0	0	
RB08	R	4	0	4	4	4	4	4A	0	4	0	4CA	Diffuse conjunctival and corneal hyperplasia; conjunctiva - goblet cell loss
RB14	L	0	0	0	0	0	0	0	0	0	0	0	
RB14	R	4	4	0	4	4	0	4A	0	4	0	4A	Conjunctiva - goblet cell loss
RB16	L	0	0	0	0	0	0	0	0	0	0	0	
RB16	R	4	0	4	1	3	2	3A	0	2	3	4CA	Conjunctiva - goblet cell loss
RB01	L	0	0	0	0	0	0	0	0	0	0	0	
RB01	R	4	4	4	4	4	4	4CA	0	3	1	2CA	Conjunctiva - goblet cell loss
RB02	L	0	0	0	0	0	0	0	0	0	0	0	
RB02	R	4	0	3	4	4	2	4A	0	4	0	4A	Bacterial keratitis
RB12	L	0	0	0	0	0	0	0	0	0	0	0	
RB12	R	0	0	4	1	2	2	3CA	0	0	2	2CA	
RB03	L	0	0	0	0	0	0	0	0	0	0	0	
RB03	R	4	3	3	4	4	2	4A	0	4	0	2C	Corneal and conjunctival epithelial hyperplasia

Appendix 3. Gross pathology results for rabbits euthanized prior scheduled 16-week euthanasia

15 Oct 2002

Accession number(s)	Animal number(s)		Protocol	Investigator(s)	Slides
			Diagnostic	Bossone	52
Species/Breed	**Sex**	**Date received**		**Necropsy date**	**Tissues**
Rabbit/New Zealand white	F	NA		15-28 May 2002	52

History: This group of rabbits was exposed to HD, corneal rupture occurred unexpectedly. Animals were euthanized.

Gross finding(s): Ruptured cornea with and without lens prolapse and loss.

Rabbit # 6. Microscopic diagnosis(ses): 02-0463 1. Eye, right: Corneal rupture with iris prolapse, lens loss, and heterophilic anterior uveitis.
2. Eye, right, coroid: Congestion diffuse severe.
3. Eye Left: Normal.

Comment: The corneal epithelium had grown around the end of the ruptured cornea, suggesting a minimum of 24 hrs between rupture and euthanasia.

Rabbit # 7. Microscopic diagnosis(ses): 02-0464 1. Eye right: Corneal rupture with transmural necrosis, granulation tissue formation, anterior synechia, and heterophilic keratitis and anterior uveitis.
2. Eye, right, conjunctiva: Conjunctivitis, chronic acute, diffuse moderate, with multifocal dermal fibrin and loss of goblet cells.
3. Eye Left: Normal.

Comments: None

Rabbit # 8. Microscopic diagnosis(ses): 2-0465 1. Eye, right: Corneal rupture with anterior synechia abundant epithelial hyperplasia, and heterophilic keratitis and anterior uveitis.
2. Eye right, conjunctiva: Conjunctivitis, diffuse, chronic-active, with dermal fibrin, epithelial hyperplasia and goblet cell loss.
3. Eye right, coroid: Congestion, diffuse, severe.
4. Eye Left: Normal.

Comments: Profound attempts to reepithelialize the damaged areas of cornea. There is almost no inflammation in the uvea.

Rabbit # 14. Microscopic diagnosis(ses): 02-0368 1. Eye, right: Corneal necrosis and rupture with heterophilic anterior uveitis, anterior synechia and widespread ulceration.
2. Eye right, conjunctiva: Conjunctivitis, diffuse, heterophilic, with dermal fibrin, epithelial hyperplasia and goblet cell loss.
3. Lung: Bronchopneumonia, heterophilic, multifocal, moderate with gram positive coccobacilli.
4. Liver: Hepatitis, multifocal, heterophilic, periportal and random
5. Trachea; esophagus; thyroid gland; Heart; left eye: Normal

Comments: The bronchopneumonia is very focal affecting only one consolidated lobe; culture was unsuccessful.

Rabbit # 16. Microscopic diagnosis(ses): 02-0469 1. Eye, right: Corneal ulceration and attenuation and severe heterophilic keratitis and anterior uveitis.
2. Eye right, conjunctiva: Conjunctivitis, diffuse, chronic-active, with dermal fibrin and goblet cell loss.
3. Eye Left: Normal.

Comments: The cornea does not appear to have ruptured, but is very near rupture.

Rabbit # 1. Microscopic Diagnosis (ses): 02-480 1. Eye, right: Corneal necrosis and rupture with heterophilic anterior uveitis, anterior synechia and epithelial hyperplasia.
2. Eye right, conjunctiva: Conjunctivitis, multifocal, heterophilic, moderate, with dermal fibrin, epithelial hyperplasia and goblet cell loss.
3. Eye Left: Normal.

Comment: Epithelial hyperplasia is attempting to reepithelialize ruptured cornea, suggesting at least 24 to 48 hours between rupture and necropsy.

Rabbit # 2. Microscopic Diagnosis (ses): 02-0481 1. Eye, right: Corneal necrosis with heterophilic keratitis and anterior uveitis, anterior synechia, numerous bacterial colonies, and goblet cell metaplasia.
2. Eye right, conjunctiva: Conjunctivitis, heterophilic, multifocal, mild, with dermal fibrin, epithelial hyperplasia and goblet cell loss.
3. Eye Left: Normal.

Comments; none

Rabbit # 12. Microscopic Diagnosis (ses): 02-0482 1. Eye, right: Corneal necrosis with heterophilic keratitis and epithelial hyperplasia with hyperkeratosis.
2. Eye right, conjunctiva: Conjunctivitis, chronic active, multifocal, mild.
3. Haired skin, eyelid: Dermatitis, ulcerative and heterophilic, focal, mild.
4. Eye Left: Normal.

Comments: The cornea is not ruptured.

Rabbit # 3. Microscopic diagnosis(ses): 2-0502 1. Eye, right: Corneal rupture with anterior synechia abundant epithelial hyperplasia, and heterophilic keratitis and anterior uveitis.
2. Eye right, conjunctiva: Conjunctivitis, diffuse, chronic-active, with epithelial hyperplasia and goblet cell loss.
3. Eye Left: Normal.

Comments: Profound attempts to reepithelialize the damaged areas of cornea.

Consolidated Comments: Many of the eyes have significant epithelial hyperplasia with loss of conjunctival goblet cells. The associated loss of mucous could exacerbate the corneal damage. Mucous replacement might be a therapeutic consideration. There is a distinct absence of inflammation in the posterior compartment. The location and type of inflammation and epithelial hyperplasia could be treatment related.