The Effects of Phosphate Buffered Saline on Corneal Chemical Alkali Burns

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Abstract: *Background:* Treatments administered after a corneal burn injury can have long term implications on wound healing. Initial treatments can include the administration of lubricants, anti-inflammatories, and antibiotic eye drops, many of which contain phosphate buffered saline (PBS) as a vehicle. As a result, the purpose of this study was to observe the effects of the commonly used PBS ophthalmic drop vehicle on corneal alkali burns over 30 days when treated 4X daily for the first 14 days.

Materials and Methods: Corneal alkali burns 5.5 mm in diameter were created on 10 anesthetized, male New Zealand White rabbits period. Animals (5) with corneal wounds were treated with PBS 4X daily or left untreated (5). Control animals (5) were not wounded, but were treated with PBS drops 4X daily. Corneal alkali burns were created by centrally placing NaOH (1N) soaked filter paper onto the cornea for 30 seconds. After removal of the filter paper, the surface of the eye was immediately debrided with a Weck-Cel® sponge and irrigated with 15 ml of sterile buffered salt solution (BSS). At days 0, 3, 7, 14 and 30 post-chemical alkali burn creation, eyes were evaluated by white light imaging and fluorescein staining. On days 14 and 30, corneal thickness was assessed via optical coherence tomography (OCT).

Results: A significant decrease in fluorescein staining indicating a significant increase in wound healing was observed on day 7 ($P< 0.05$) in the PBS treatment group when compared to the non-treated corneal alkali burns. A significant increase in fluorescein staining or decrease in wound healing was observed on day 30 ($P< 0.0005$) in the PBS treatment group when compared to non-treated corneal alkali burns. White light imaging and OCT results, respectively, showed no significant differences in corneal opacity or corneal swelling between treatment groups at any time point.

Conclusions: This study suggests that the treatment of corneal alkali burns with ophthalmic solutions containing PBS may impact wound closure while having no significant effect on corneal opacity or corneal swelling.

Keywords: Corneal epithelial wound healing, re-epithelialization.

1. INTRODUCTION

During Operations Iraqi Freedom (OIF), Enduring Freedom (OEF), and New Dawn (OND), 92% of the 8,824 evacuated military members sustained head or facial injuries from explosive devices [1]. Of the 3,008 blast eye injuries reported, 50% were open wounds, 48% were contusion, and 2% were burns or nerve injuries. In addition to these injuries, military members are also at risk for chemical injuries on the battlefield and in garrison, which make up 11.5% to 22.1% of ocular injuries including the civilian population [2]. For military members with ocular injuries in OIF, OEF, and OND, 65% of these eye injuries were designated level III in severity, which are injuries that are very likely to result in vision loss [1]. To prevent vision loss as a result of these severe injuries, immediate and proper treatment is crucial. This includes the administration of topical lubricants, anti-inflammatories, and antibiotics at often an exaggerated dosing. Unfortunately, for many of these injuries, treatment options are frequently limited and the initial treatments administered could have implications on both the initial and long term healing process [3-5]. Phosphate buffered saline (PBS) is a commonly used vehicle in many of these ophthalmic treatments. To determine the effects of PBS on corneal alkali burns, we sought to study the long-term progression of corneal alkali burns over 30 days when treated 4Xdaily with a topical treatment of PBS.

For those with chemical ocular injuries, established treatment regimens recommend that the eyes be irrigated often with balanced salt solution (BSS) at least 30-60 minutes in an attempt to dilute the chemicals absorbed by the cornea. It is imperative that chemical agents be irrigated as efficiently and quickly as possible with the proper rinsing agent as chemical

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alkali agents penetrate the corneal stroma, destroying proteoglycans and collagens [6]. Alkali burns are especially caustic; the lipophilic properties of these agents allow for a more rapid penetration of tissues than acidic agents, leading to necrosis and ischemia [6]. The complete irrigation of these injuries can take as many as 20 liters of fluid, which is essential to ensure removal of the reagent. The pH of the tear film is checked to ensure the complete removal of the agent [3]. After the initial irrigation of the eye, it is critical that measures be taken quickly to resolve any damage sustained to the cornea or conjunctival epithelium in order to prevent scarring. For these types of injuries, the therapeutics available to treat and promptly aid in the repair of these ocular tissues can be limited. The recommended standard of care includes antibiotic drops used in conjunction with topical steroids to reduce inflammation. Vitamin C drops to promote collagen remodeling, cycloplegic drops for ciliary pain and spasm, and acetylcysteine drops as a collagenase inhibitor may also be administered [3].

As drops are the most frequent form of administration of ocular therapeutics, the chemical composition of drops used on these injuries should be taken into consideration. The type of treatment administered to the eye has impacts on the entire healing environment. The use of phosphates and phosphate buffers like PBS in ophthalmic drops and artificial tears may influence the outcome of these injuries. As the corneal epithelium is greatly compromised, the treatments utilized on the cornea may alter the entire ocular and corneal environment and affect corneal healing. Some studies have demonstrated that human corneal–limbal epithelial (HCLE) and human conjunctival epithelial (HCjE) cells treated with 100 mM of phosphate buffer, exhibit decreases in cell viability [7]. This means that PBS treatment may be detrimental to the health of the cell, specifically corneal and conjunctival epithelial cells. As a result, we investigated the consequence of administering frequent dosing (4X daily) PBS drops over a 14 day period after corneal epithelial injury to determine how this would affect the outcome of chemical alkali injuries. While other corneal chemical alkali injuries have been investigated in rabbits, these studies are often terminated by day 14, whereas we observed these burns for 30 days. This study demonstrates the pathology of severe chemical burns over time, including how treatment with PBS drops when utilized as a therapeutic vehicle and or excipient may affect corneal alkali burn pathology.

2. MATERIALS AND METHODS

2.1. Animals

Male New Zealand White (NZW) rabbits (2.5-4.0 kg) were obtained from Charles River Laboratories (Wilmington, MA). Animals were randomly grouped (N=5 per group). All animal procedures were performed on anesthetized animals. After anesthetization, a corneal alkali burn was created on the left eye of 10 animals, while 5 animals served as uninjured controls. Of the animals with corneal alkali burns, 5 received treatment 4X daily with 1 drop (50 μl) of phosphate buffered saline (PBS; Gibco, Grand Island, NY, USA) for 14 days post injury and 5 animals received no treatment. Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of the Guide for the Care and Use of Laboratory Animals, National Research Council. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by AAALAC International. This study was also conducted in compliance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Nictitating Membrane Removal

Nictitating membrane removal occurred three weeks prior to corneal alkali burn creation. Ketamine hydrochloride (VetOne®, Boise, ID USA; 35-45 mg/kg), buprenorphine SR-LAB (0.5 mg/kg), and xylazine (5 mg/kg) were administered via intramuscular (IM) injection for induction before nictitating membrane removal surgery. Animals were placed on isoflurane (1 to 3.5%) and the left eyes of the rabbits were anesthetized with 0.5% tetracaine hydrochloride (Alcon, Ft. Worth, TX, USA). A 5% povidone iodine solution (10% povidone-iodine; Purdue Pharma L.P., Stamford, CT, USA) in PBS was applied to sterilize the surgical site. A topical antibiotic drop, 0.1% moxifloxacin (Alcon) was administered, followed by the placement of an eyelid speculum and sterile draping. A 0.5% tetracaine hydrochloride drop (Alcon) was then administered for a second time. Forceps (#12) gripped the nictitating membrane and a needle (30G) was used to inject 0.1-0.2 ml 2% lidocaine hydrochloride with epinephrine 1:100,000 USP (Hospira, Inc. Lake Forest, IL, USA) into the posterior and anterior nictitating membrane base. The nictitating membrane was removed using Westcott scissors and a Weck-Cel® sponge was used to remove any blood. High temperature cautery was employed to close the surgical site and Bacitracin Zinc and Polymixin B Sulfate Ophthalmic Ointment USP (Bausch & Lomb, Rochester, NY, USA) was applied to the surgical site. Animals were placed in an oxygen chamber (39-40% oxygen) for recovery and checked twice daily for three days. The animals were treated for pain as necessary and given three weeks to heal from surgery.

2.3. Corneal Alkali Burn Creation

Grade 52, 175 μm thick, 0.015% ash, circular filter paper 5.5 mm in diameter (Whatman®, GE Healthcare Bio-Sciences, and Pittsburg, PA, USA) was soaked in 1N NaOH for 60 seconds. The filter paper was placed on the central cornea, which was determined by caliper measurements, for 30 seconds. Burns were immediately and liberally rinsed with 15 ml BSS (Baxter, Deerfield, IL, USA) and debrided. Following corneal alkali injury, the injured eyes were either left untreated or treated 4X daily for 14 days with one drop of PBS.

2.4. Corneal Imaging

Images were obtained on days 0, 3, 7, 14, and 30 post-injury utilizing a camera (7D, Canon USA., Melville, NY, USA) with a 100 mm macro lens. Corneal wounds were visualized via fluorescein uptake immediately after the creation of the injury. Fluorescein sodium ophthalmic films USP (Fluorets®, Chauvin Laboratory, Aubenas, France) were moistened with 100 μl sterile BSS and the solution was placed on the eye for approximately 10 seconds before rinsing with BSS. The corneal injuries were imaged under a cobalt blue light. Using ImageJ software (U.S. National Institutes of Health, Bethesda, MD, USA) as previously described [8], wound healing percentages and areas of corneal opacity were quantified by three masked observers. Time points were compared to the original wound at 0 hours. Optical coherence tomography (OCT, Bioptigen Spectral Domain Optical Coherence Tomography (SD-OCT)) images were also acquired and analyzed with ImageJ software. Measurements were taken at the widest section of the central cornea [9].

2.5. Statistical Analysis

Data sets were analyzed using a repeated measures 2-way analysis of variance (ANOVA) with GraphPad Prism 7.01 (GraphPad Software Inc., La Jolla, CA, USA). Independent values are reported as mean ± the standard error of the mean (SEM). A *P*-value of <0.05 was deemed significant for statistical analyses.

3. RESULTS

3.1. PBS Exhibits Modest but Significant Effects on Re-Epithelialization of Alkali Burns

Corneal epithelial wound closure was measured in injured rabbit eyes over time via fluorescein staining and expressed as a percent wound healing compared to the initial wounded area created on day 0. PBS treated and non-treated corneal alkali burns showed no differences in the percentage of wound healing at day 3 with both groups demonstrating almost 100% epithelial closure. By day 7 there was a significant difference ($P < 0.05$) in wound healing in the untreated group at 96% compared to the PBS treated group at 98%. On day 14, again PBS treated eyes were 98% healed healing versus 97% in untreated injuries. By day 30, a significant difference ($P < 0.0001$) was found between the PBS treated group at 87% wound closure versus the untreated group at 92% wound closure.

3.2. No Changes in the Area of Corneal Opacity with PBS Treatment

Areas of corneal opacity were measured via ImageJ post-injury creation on days 0, 3, 7, 14, and 30. The difference in areas of corneal opacity between the PBS treated injuries and untreated injuries was found to increase over time when compared to day 0. PBS treated and untreated injuries both showed a 12% decrease in corneal opacity on day 7. On day 14, PBS treated injuries showed a 32% decrease in the area of corneal opacity and untreated injuries were found to have a 38% decrease in the area of corneal opacity. By day 30, the difference between the PBS treated injuries and untreated injuries had increased. PBS treated injuries showed a 54% decrease in corneal opacity and untreated injuries had a greater decrease in corneal opacity at 61%. PBS treated injuries displayed larger areas of corneal opacity over time; however, no significant differences were found between PBS treated and untreated injuries. While the areas of corneal opacity were decreased by day 30, areas of opacity were still present in both the PBS treated and untreated groups.

3.3. Corneal Alkali Burns Exhibit Significant Increases in Swelling 30 Days Post-Injury

SD-OCT imaging results demonstrated that PBS treated and untreated injured eyes showed significant (****$P < 0.0001$) corneal thickening compared to uninjured eyes at days 14 and 30. On day 14 post-injury, the average corneal thickness of PBS treated

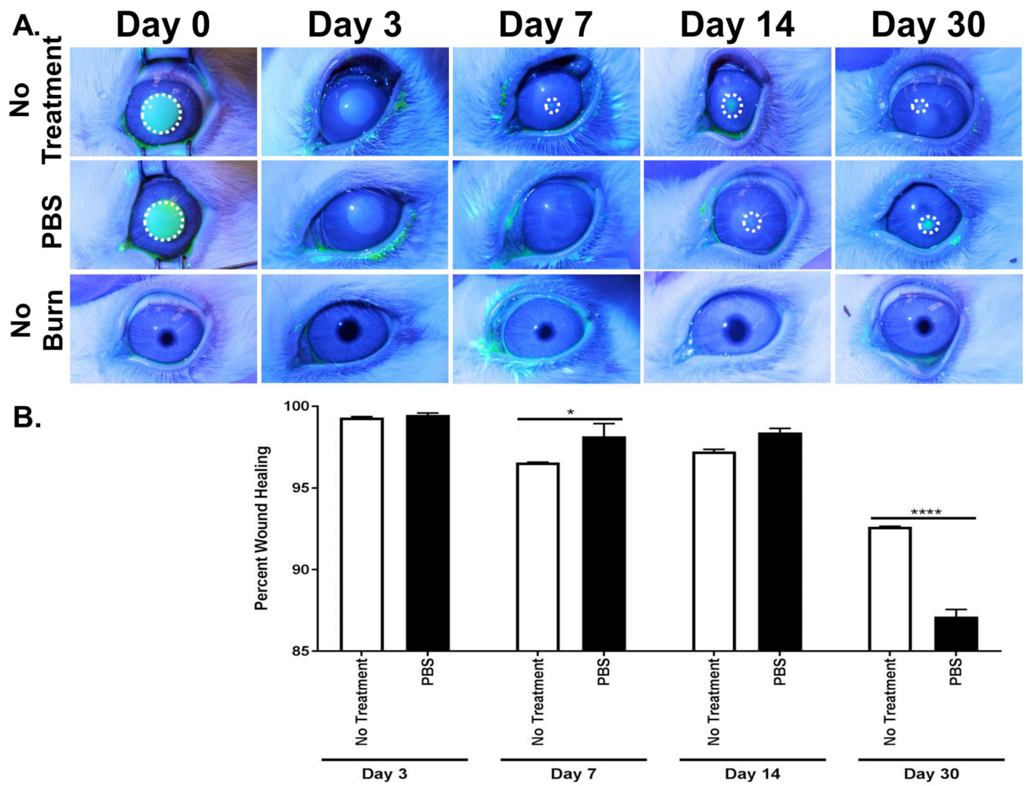

Figure 1: PBS treatment affects epithelial wound closure. At day 0, 3, 7, 14 and 30 post chemical alkali burn creation and treatment with PBS, eyes were evaluated by fluorescein staining. Representative images of fluorescein staining (**A**) show the area of corneal epithelial wound closure of non-treated wounds and those treated with PBS 4X daily. The histogram (**B**) represents the percentage of wound closure at day 0, 3, 7, 14 and 30 post chemical alkali burn creation and treatment with PBS. The values are expressed as mean ± SEM and are representative of three blinded observations of 5 animals per group. The comparisons of the percentage areas of fluorescein staining in PBS treated versus non-treated wounds were performed by utilizing a 2-way repeated measures ANOVA. *$P < 0.05$, ****$P < 0.0001$.

injuries was 797 μm ± 26 while untreated injuries were 749 μm ± 78. Uninjured eyes remained at 380 μm ± 11. By day 30 post-injury, corneal thickness was nearly double in both PBS treated (848 μm ± 75) and untreated injured (800 μm ± 87) groups compared to uninjured eyes (388 μm ± 11) (****$P < 0.0001$). The difference between the PBS and non-PBS treated groups was not clinically nor statistically significant.

4. DISCUSSION

Military members are faced with the constant threat of corneal chemical injuries both in garrison and on the battlefield. The immediate treatment of these injuries is crucial to the prevention of permanent vision loss. Current treatments for corneal chemical burns are limited, and the type of urgent care administered could have detrimental initial and long-term vision outcomes if not appropriate. Previous studies have shown that the use of PBS as a buffer in ophthalmic drops has been associated with band keratopathy or calcification of the cornea [4,5]. Other studies suggest that rinsing corneal burns with copious amount of borate buffer versus saline or phosphate buffers may more efficiently neutralize the pH of the eye [4,5]. The types of buffers used as rinsing agents and the types of buffers used in ophthalmic drops may influence long term wound healing. Given the use of phosphate buffers as a potential rinsing agent and the prevalence of PBS in ophthalmic drops, we investigated the application of PBS drops 4X daily for 14 days on corneal alkali burns.

As the purpose of this study was to assess the progression of corneal alkali burns, corneal epithelial re-epithelialization, areas of corneal opacity, and corneal thickness, the injured eyes were observed for 30 days after initial burn creation and treated with PBS 4X daily for the first 14 days post-injury. Our results revealed significant differences in corneal epithelial wound healing between PBS treated groups and untreated groups over time. Some differences on average, although not significant, were noted between the treatment groups in regards to the areas of corneal opacity. When considering the opacification of the cornea, slightly larger areas on average of corneal opacity were seen on days 14 and 30 in the PBS

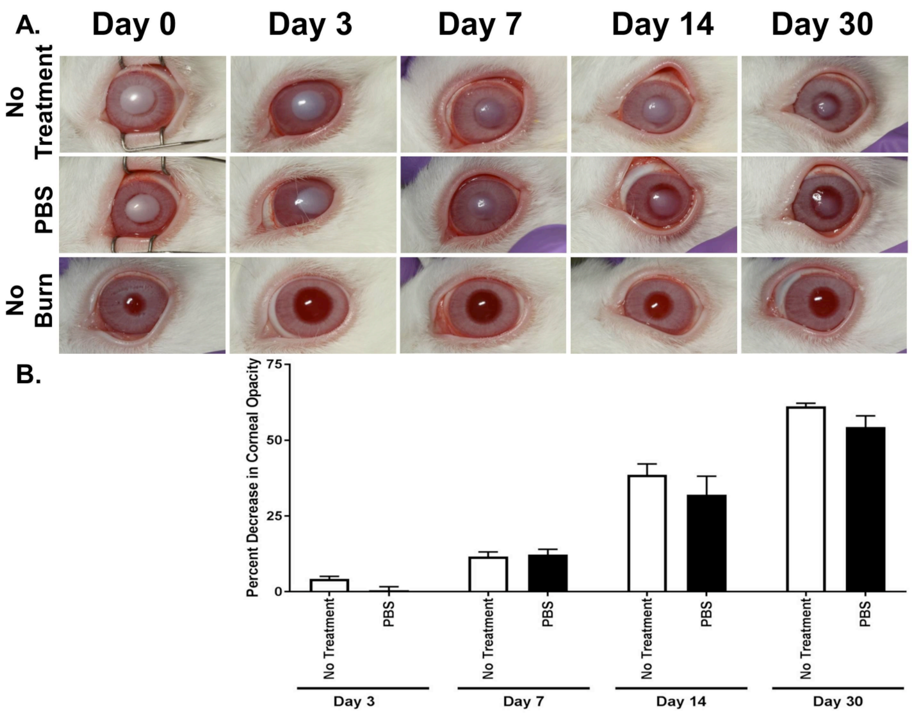

Figure 2: The effect of corneal burns on corneal opacity. On day 0, 3, 7, 14 and 30 post chemical alkali burn creation and treatment with PBS, eyes were evaluated by white light imaging. Representative images, (**A**) show the area of corneal opacity of non-treated wounds and those treated with PBS 4X daily. The histogram (**B**) represents the percentage of decrease in corneal opacity at day 0, 3, 7, 14 and 30 post chemical alkali burn creation and treatment with PBS. The values are expressed as mean ± SEM and are representative of three blinded observations of 5 animals per group. Comparisons of the percentage areas of corneal opacity in PBS treated versus non-treated wounds were performed by utilizing a 2-way repeated measures ANOVA.

treated injuries versus the untreated injuries. This could indicate that PBS treatment may slow the rate at which corneal opacity resolves. The larger areas of corneal opacity in the PBS treated groups on day 30 could also be linked to the decrease in re-epithelialization leaving the cornea exposed to inflammatory mediators and enzymes in the tear film. There were essentially no differences in the corneal thickness between the treatment groups.

The reappearance of a corneal epithelial defect after the epithelium has initially closed is common and these types of injuries can result in corneal scarring, recurrent corneal erosion, corneal opacity, ulceration, and neovascularization [10]. In fact, corneal epithelial burns are typically divided into four clinical phases of healing: immediate (day 0), acute (days 1-7), early reparative (days 8-20), and late reparative (> 20 days) [11]. These injuries were observed well into the late reparative phase, which normally occurs on >20 days post injury. During the late reparative stage, the injury will either be resolved or complications will persist [10]. In this model, the corneal epithelium appeared to re-erode in both treatment groups, with the PBS treated wounds exhibiting significantly smaller defects on day 7, but significantly larger defects on day 30, when compared to untreated wounds. The positive impact of PBS on wound healing at day 7 indicates a positive wound healing environment. The results at day 7 also reflect a different status of the ocular environment and corneal epithelium than what is observed on day 30. By following these corneal epithelial burns out to 30 days, we were able to observe a complex interaction of environmental factors, which are not identical between treatment groups. More importantly, the requirement to sustain and maintain a pro-healing environment is different between treatment groups due to the initial 14 day treatment with PBS. This means that the initial treatment applied to a corneal alkali burn could have long-term effects. In this study, PBS wounds healed more efficiently up to day 14 before the treatment was terminated. These results could indicate that terminating a topical lubricating treatment after 14 days may have detrimental effects on wound healing and lead to recurrent erosions. These results could also suggest that the properties of PBS were beneficial to wound healing. In either case, treatment with PBS drops could have a minimally beneficial effect on wound healing and the wound healing environment up to 14 days in a corneal alkali burn injury. Taken together, this study demonstrates that PBS as a topical

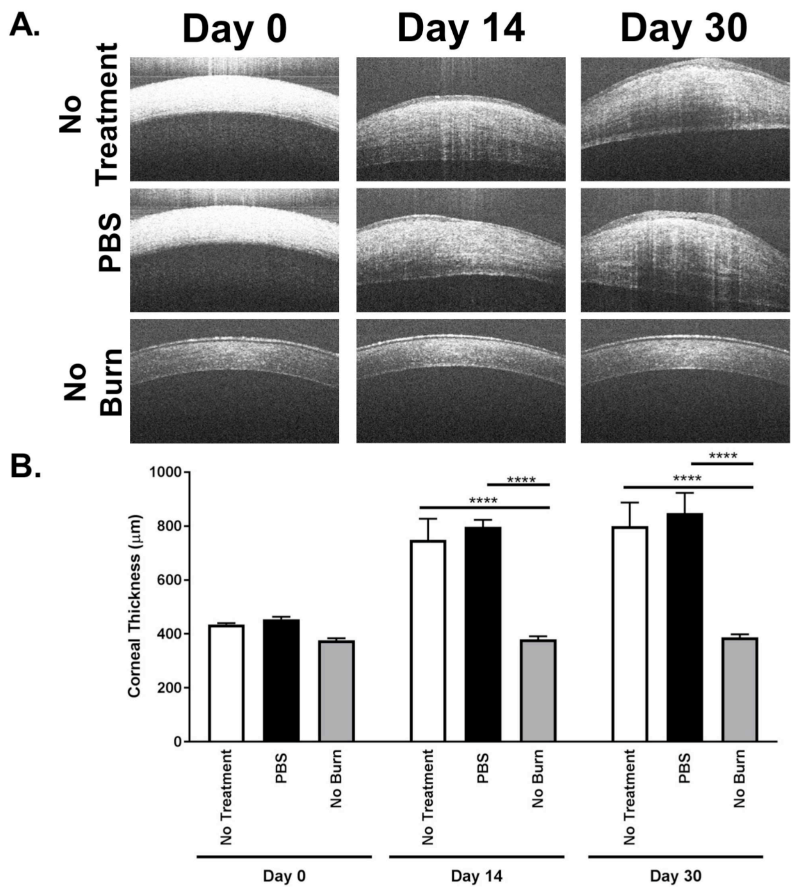

Figure 3: The effect of corneal burns on corneal swelling. Representative SD-OCT images (**A**) were taken immediately after the corneal alkali burn injury creation at 0 hours and on days 14 and 30 post injury of non-treated wounds, PBS treated wounds, and non-injured eyes. The histogram (**B**) represents corneal thickness values as mean ± SEM and representative of 5 animals per group. Comparisons between the treatment groups were made utilizing a 2-way repeated measures ANOVA. ****$P <$ 0.0001.

treatment did not induce negative effects on re-epithelialization, corneal opacity, or corneal swelling. After termination of PBS treatment, however, wounds were significantly larger at 30 days when compared to burns receiving no treatment indicating that the initial treatment with PBS could affect long term wound closure and the ocular environment if discontinued too soon.

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REFERENCES

[1] Lo M, Davis S, LTC Capó-Aponte J and LTC Cast K. Retrospective Case Series of US Service Members Medically Evacuated from Operations Enduring Freedom, Iraqi Freedom, and New Dawn with Blast-Related Head and Facial Injuries. EC Neurology 2016: 402-11.

[2] Sharma N, Kaur M, Agarwal T, Sangwan VS and Vajpayee RB. Treatment of acute ocular chemical burns. Surv Ophthalmol 2017. https://doi.org/10.1016/j.survophthal.2017.09.005

[3] Mazzoli R. Now See This: Care for Ocular Chemical Injuries. Frontlines of Eye Care 2017.

[4] Rihawi S, Frentz M, Reim M and Schrage NF. Rinsing with isotonic saline solution for eye burns should be avoided. Burns 2008; 34: 1027-32. https://doi.org/10.1016/j.burns.2008.01.017

[5] Rihawi S, Frentz M and Schrage NF. Emergency treatment of eye burns: which rinsing solution should we choose? Graefes Arch Clin Exp Ophthalmol 2006; 244: 845-54. https://doi.org/10.1007/s00417-005-0034-3

[6] Fish R and Davidson RS. Management of ocular thermal and chemical injuries, including amniotic membrane therapy. Curr Opin Ophthalmol 2010; 21: 317-21. https://doi.org/10.1097/ICU.0b013e32833a8da2

[7] Schuerer N, Stein E, Inic-Kanada A, Pucher M, Hohenadl C, Bintner N, et al. Implications for Ophthalmic Formulations: Ocular Buffers Show Varied Cytotoxic Impact on Human Corneal-Limbal and Human Conjunctival Epithelial Cells. Cornea 2017; 36: 712-8. https://doi.org/10.1097/ICO.0000000000001199

[8] Griffith GL, Kasus-Jacobi A, Lerner MR and Pereira HA. Corneal wound healing, a newly identified function of CAP37, is mediated by protein kinase C delta (PKCdelta). Invest Ophthalmol Vis Sci 2014; 55: 4886-95. https://doi.org/10.1167/iovs.14-14461

[9] Muscat S, Parks S, Kemp E and Keating D. Repeatability and reproducibility of macular thickness measurements with the Humphrey OCT system. Invest Ophthalmol Vis Sci 2002; 43: 490-5.

[10] Eslani M, Baradaran-Rafii A, Movahedan A and Djalilian AR. The ocular surface chemical burns. J Ophthalmol 2014; 2014: 196827. https://doi.org/10.1155/2014/196827

[11] Singh P, Tyagi M, Kumar Y, Gupta KK and Sharma PD. Ocular chemical injuries and their management. Oman J Ophthalmol 2013; 6: 83-6. https://doi.org/10.4103/0974-620X.116624

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