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Treatment of corneal chemical alkali burns with a crosslinked thiolated hyaluronic acid film

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ABSTRACT

Purpose: The study objective was to test the utilization of a crosslinked, thiolated hyaluronic acid (CMHA-S) film for treating corneal chemical burns.
Methods: Burns 5.5 mm in diameter were created on 10 anesthetized, male New Zealand white rabbits by placing a 1N NaOH soaked circular filter paper onto the cornea for 30 s. Wounds were immediately rinsed with balanced salt solution (BSS). CMHA-S films were placed in the left inferior fornix of five injured and five uninjured animals. Five animals received no treatment. At 0 h, 48 h, 96 h, and on day 14 post chemical burn creation, eyes were evaluated by white light imaging, fluorescein staining, and optical coherence tomography (OCT). Corneal histology was performed using H&E and Masson's Trichrome stains.
Results: Image analysis indicated biocompatible CMHA-S treatment resulted in significant decreases in the areas of corneal opacity at 48 h, 96 h, and on day 14 postoperatively. A significant increase in re-epithelialization was seen 14 days post injury. CMHA-S treated corneas showed significantly less edema than untreated burns. No pathological differences were observed in corneal histological samples as a result of CMHA-S treatment.
Conclusions: CMHA-S films facilitate re-epithelialization and decrease the area of corneal opacity in our corneal alkali burn rabbit model.

Abbreviations: ANOVA, analysis of variance; ARVO, The Association for Research in Vision and Ophthalmology; CMHA-S, crosslinked carboxymethylated thiolated hyaluronic acid; CRADA, cooperative research and development agreement; BSS, balanced salt solution; HA, hyaluronic acid; H&E, hematoxylin and eosin; HOA, high order aberration; LASEK, laser-assisted subepithelial keratomileusis; MRMC, medical research and materiel command; NZW, New Zealand White; OCT, optical coherence tomography; ORISE, Oak Ridge Institute for Science and Education; PAD, program area directorate; PBS, phosphate buffered saline; PEGDA, polyethylene (glycol) diacrylate; SEM, standard error of the mean.

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1. Introduction

Regardless of occupational safety measures, clinicians still treat a number of ocular surface injuries caused by heat, acids, or alkali agents. Clinicians also treat patients with severe facial and eyelid burns that result in devastating ocular injuries leading to vision loss and/or blindness [1]. Not only are these injuries still prevalent with alkali or acidic agents representing 11.5–22.1% of ocular traumas [2], chemical and thermal injuries are some of the most clinically difficult to treat [3]. In the case of chemical burns, alkali burns are more severe and caustic as alkali agents penetrate the ocular tissues more rapidly due to lipophilic properties. This results in tissue necrosis and ischemia which often gives the eye a misleading, but quiescent white appearance [4]. From a molecular standpoint, exposure to alkali compounds causes the saponification of fatty acids in cell membranes as they penetrate the corneal stroma and destroy proteoglycans and collagens [4]. As corneal alkali burns heal, the injured tissues secrete proteolytic enzymes resulting in additional and continuous tissue damage [4]. For these reasons, the clinical rehabilitation of corneal alkali burn patients is often very challenging given long term complications due to reoccurring corneal epithelial erosions and chronic inflammation which leads to vision loss [5]. Patients with devastating facial burns frequently have damage to the periorbital tissues (i.e. conjunctiva, periorbital fat, glands and eyelids) that can indirectly lead to similar outcomes as a direct insult. Regardless if the patient is suffering from chemical or thermal burn injury, the vision loss that can occur due to these injuries is the same when the ocular surface is not healed in a timely manner. As a result, clinicians are in need of more advanced therapeutics to treat these patients.

While amniotic membrane, buccal skin grafts, tarsorrhaphies (partial suturing of the eyelids), and bandage contact lenses are available as therapies, these treatments fail in treating the most severe ocular burn patients. In severe injuries, a topical treatment such as ascorbate drops, citrate drops, corticosteroids, or bandage contact lenses may be utilized in conjunction with amniotic membrane transplantation, limbal stem cell transplantation, or a corneal transplant to resolve any corneal scarring or opacity [4]. When a stable ocular surface cannot be restored via limbal stem cell transplantation, a keratoprosthesis placement may be required. Despite interventions, however, suboptimal outcomes still frequently occur. Furthermore, the reliance on topical eye drop administration is not sufficient. Topical ophthalmic drops dissipate almost completely after drop administration [6,7] and must also be applied frequently. This is a problem for burn patients or elderly patients who might not be able to place a drop without assistance. A treatment that eliminates drop administration would not only be an improved treatment option, but would also decrease the burden of care. As a result, there is a need for more sophisticated ophthalmic treatment modalities that eliminate ophthalmic drops as well as promote wound healing. To this end, Jade Therapeutics, Inc. (wholly owned subsidiary of EyeGate Pharmaceuticals, Salt Lake City, UT, USA) has developed a continuous drug delivery system that would eliminate the need for drop administration while promoting wound healing. This drug delivery system is a proprietary thiolated, crosslinked hyaluronic acid (CMHA-S) polymer for ocular use that has been demonstrated to accelerate wound closure following photorefractive keratectomy (manuscript under review). Furthermore, when applied as a liquid gel, this polymer has a residence time on the ocular surface of longer than two hours (manuscript under review) due to unique engineering. This polymer, however, can also be manufactured as a thin, flexible film which can be inserted into the inferior fornix to provide sustained release of therapeutic agents. Crosslinked CMHA-S is a versatile, biocompatible polymer that combines the documented wound healing properties of HA [8] with drug-delivery capabilities to provide an innovative ophthalmic treatment when installed quickly (without the use of suturing or tissue glue) directly to the ocular surface [9]. This type of treatment not only has the potential to provide prolonged therapeutic delivery and promote ocular tissue repair, but also would not rely on self-administration making it extremely beneficial for ocular burn management. Due to the intrinsic healing properties of this polymer, we investigated this novel CMHA-S treatment modality to treat one of the most challenging types of ocular chemical burns, alkali chemical injures. We hypothesize that crosslinked CMHA-S films are not only safe, but can also provide for the advanced treatment of corneal alkali burns as demonstrated in our ocular alkali chemical model of wound healing.

2. Materials and methods

2.1. Animals

Male New Zealand White (NZW) rabbits (2.5–4.0 kg) were purchased from Charles River Laboratories (Wilmington, MA) and randomly grouped (N=5 per group). Sample-size requirement estimates are based on clinical examination scores and testing at the study endpoint. The sample size was estimated using a power analysis (SigmaPlot v12.5). Three treatment groups consisted of animals that received CMHA-S films on uninjured eyes, animals that received CMHA-S films with alkali burns, and animals that received no treatment with CMHA-S films, but had corneal alkali burns. All animal procedures were performed on anesthetized animals. Protocols were reviewed and approved by the United States Army Institute of Surgical Research (USAISR) Institutional Animal Care and Use Committee (IACUC). This study was conducted in compliance with Animal Welfare Regulations, other Federal statutes relating to animals and experiments involving animals, and the principles set forth in the Guide for the Care and Use of Laboratory Animals, National Research Council.

2.2. Nictitating membrane removal

The nictitating membrane was removed three weeks prior to corneal alkali burn creation or film placement. Prior to nictitating membrane removal, 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 and animals were placed on isoflurane (1–3.5%). Left eyes of the rabbits were anesthetized

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