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Mechanisms of Retinal Damage after Ocular Alkali Burns

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Q5 Alkali burns to the eye constitute a leading cause of worldwide blindness. In recent case series, corneal transplantation revealed unexpected damage to the retina and optic nerve in chemically burned eyes. We investigated the physical, biochemical, and immunological components of retinal injury after alkali burn and explored a novel neuroprotective regimen suitable for prompt administration in emergency departments. Thus, *in vivo* pH, oxygen, and oxidation reduction measurements were performed in the anterior and posterior segment of mouse and rabbit eyes using implantable microsensors. Tissue inflammation was assessed by immunohistochemistry and flow cytometry. The experiments confirmed that the retinal damage is not mediated by direct effect of the alkali, which is effectively buffered by the anterior segment. Rather, pH, oxygen, and oxidation reduction changes were restricted to the cornea and the anterior chamber, where they caused profound uveal inflammation and release of proinflammatory cytokines. The latter rapidly diffuse to the posterior segment, triggering retinal damage. Tumor necrosis factor-α was identified as a key proinflammatory mediator of retinal ganglion cell death. Blockade, by either monoclonal antibody or tumor necrosis factor receptor 1 and 2 gene knockout, reduced inflammation and retinal ganglion cell loss. Intraocular pressure elevation was not observed in experimental alkali burns. These findings illuminate the mechanism by which alkali burns cause retinal damage and may have importance in designing therapies for retinal protection. *(Am J Pathol 2017, ■: 1–16; http://dx.doi.org/10.1016/j.ajpath.2017.02.005)*

Q6 Alkali burns may cause significant corneal scarring and blindness even if promptly treated.[1–4] Standard corneal transplantation can temporarily restore corneal clarity, but long-term results have been disappointing.[5] Burn-induced loss of limbal epithelial stem cells that regenerate corneal epithelium complicates surface healing.[6,7] Implantation of an artificial cornea can restore transparency. In many patients, the clear ocular media reveals the presence of optic nerve pallor and cupping, characteristic of retinal degeneration and severe glaucoma.[8] The mechanism of the damage to the posterior eye is not clear, but previous reports postulated that the alkali diffuses posteriorly and directly damages the retina.[9] Inflammatory intraocular pressure (IOP) elevation has also been implicated.[10] However, in a recent study on corneal alkali burns in rabbits (*in vivo*) and porcine eyes (*ex vivo*), direct pH determination performed in the vitreous revealed an unchanged, normal pH up to 6 hours after the burn. This casts doubt on the possibility of a direct effect of the alkali on the retina. In addition, tumor necrosis factor (TNF)-α expression was shown to acutely increase in the retinas of mice, followed by retinal ganglion cell (RGC) apoptosis 24 hours after the burn.[11] This

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suggests that the damage to the retina may be mediated by immunological processes, such inflammatory cytokines, rather than by direct pH change. These findings also raise the possibility of using targeted immunomodulatory therapy, such as antibody against TNF-α, for neuroprotection. Indeed, antibody against TNF-α given soon after burn was shown to provide significant protection to both the cornea and retina.[11] The promise of neuroprotection in human eyes after alkali burn prompted the present study. The goal of this study is to elucidate the biophysical and biological processes that lead to retina damage after alkali trauma and to explore the protective role of targeted immunomodulation with an anti−TNF-α agent as a novel adjunct therapy.

Materials and Methods

Mouse Model of Alkali Burn

All animal-based procedures were performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and the NIH *Guide for the Care and Use of Laboratory Animals*.[12] This study was approved by the Animal Care Committee of the Massachusetts Eye and Ear Infirmary. C57BL/6 and TNFRSF1A1B knockout mice were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice between the ages of 6 and 12 weeks were used for this study. This model of alkali chemical burn is based on our previous study.[11] In brief, mice were anesthetized using 60 mg/kg ketamine and 6 mg/kg xylazine, and deep anesthesia was confirmed by a toe pinch test. Proparacaine hydrocloride USP 0.5% (Bausch and Lomb, Tampa, FL) eye drop was applied to the cornea for 1 minute and carefully dried with a Weck-Cel (Beaver Visitec International, Inc., Waltham, MA). A 2-mm-diameter filter paper was soaked into 1 mol/L sodium hydroxide solution for 10 seconds, dried from excess sodium hydroxide by a single touch on a paper towel, and applied onto the mouse cornea for 20 seconds. Complete adherence of the filter paper on the corneal surface was ensured by gentle push of the perimeter using forceps. After the filter paper was removed, prompt irrigation with sterile saline for 10 seconds was applied using a 50-mL syringe with a 25 G needle. The mouse was then placed on a heating pad, positioned laterally, and irrigated for another 15 minutes at low pressure using sterile saline. Buprenorphine hydrochloride (0.05 mg/kg; Buprenex Injectable; Reckitt Benckiser Healthcare Ltd, UK) was administered s.c. for pain management. A single drop of topical Polytrim antibiotic was administered after the irrigation (polymyxin B/trimethoprim; Bausch & Lomb Inc., Bridgewater, NJ). Mice were kept on a heating pad until fully awake.

Rabbit Model of Alkali Burn

New Zealand white rabbits, weighing 4 to 5 kg, were obtained from Charles Rivers (CT). Rabbits were placed on a heating pad, and anesthesia was administered using i.m. ketamine, 20 mg/kg, followed by i.p. injection of urethane, 1300 mg/kg (Sigma Aldrich, St. Louis, MO) diluted in 2-mL sterile water for injection.[13] Additional administration of urethane, 400 mg/kg, was provided every 5 hours to maintain anesthesia for 24 hours. Heart rate and temperature were continuously monitored. Alkali burn to the cornea was performed by placing an 8-mm trephine on the corneal surface and filling the trephine with 1 mL 2 mol/L sodium hydroxide for 40 seconds. The alkali was carefully absorbed from inside the trephine using a Weck-cel sponge, and the trephine was filled with 3 mL of sterile saline, which was then aspirated. The eye was then irrigated with saline solution for 20 seconds, followed by slow irrigation for 15 minutes, as described above. At the end of the experiment, an epidermal fentanyl patch was placed (12 μg/hour) for 3 days to reduce discomfort. Rabbits received twice-daily topical antibiotic ointment (erythromycin). Rabbits were euthanized at the completion of the experiment with 40 mg/kg ketamine and 10 mg/kg xylazine, followed by 100 mg/kg Fatal Plus IV injection (sodium pentobarbital).

pH, Oxygen, and Oxidation-Reduction Measurements

In vivo pH measurements were performed using two different pH probes: fiberoptic pH-1-micro with an outer diameter of 140 μm (PreSens, Regensburg, Germany) and microelectrode pH-50 with an outer diameter of 50 μm (Unisense, Aarhus, Denmark). Two different probes were used to validate the results. Briefly, the fiberoptic pH technology is based on dual-lifetime referencing technique. A fiberoptic probe is inserted in the eye and a coupled photo-emitting diode performs simultaneous excitation of a pair of luminophores, one reporting the pH and the other acting as reference.[14] The phase difference between the two excitations represents the pH of the sample and is independent of light intensity or wavelength interference. The probe has a linear response time of approximately 30 seconds at a pH range between 5.5 and 8.5 at 5°C to 50°C. At neutral pH, the probe has resolution ±0.001, accuracy ±0.05, and drift <0.05. The electrode pH sensor is based on selective diffusion of protons through pH-sensitive glass, and measures the potentials between the electrolyte and a reference electrode. The response time of the electrode is <10 seconds in the pH range 7 to 14, with resolution of 0.1 and linear response from 2 to 10 pH units from −10°C to 90°C. The sensors were precalibrated at pH 5, 7, and 10 before implantation, and the linear regression was calculated with $R^2 > 0.98$ in all cases.

In vivo oxygen measurements were performed using a 50-μm diameter glass-electrode oxygen sensor (Unisense, Aarhus, Denmark). The oxygen probe measures the diffusion of oxygen through a silicone membrane to an oxygen-reducing cathode. The reducing cathode is polarized against an internal silver/silver chloride anode, and the potential difference between the anode and cathode represents the oxygen partial pressure. The probe has linear response