Corneal Wound Healing Model in New Zealand White Rabbits for Evaluating Persistent Corneal Epithelial Defects

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Introduction
Persistent corneal epithelial defects (PCED) can be caused by chronic ocular infections, severe dry eye, neurotrophic/diabetic keratitis, chemical exposure, and exposure to blast traumas as in a military theater. Ocular surgeries can also lead to PCED conditions.

Purpose
To develop a delayed wound healing model for evaluating the corneal wound healing capabilities for PCED.

Methods
The animals were anesthetized with ketamine and xylazine given intramuscularly (IM). The epithelial defect was created in the center of the cornea with an 8.5 mm Camellin LASEK alcohol well. A 20% ethyl alcohol solution was applied for 2.5 minutes. The ocular surface outside the Camellin well was irrigated with balanced salt solution (BSS) during alcohol application. The alcohol solution was then removed and the cornea was irrigated with BSS. The epithelium was removed (to the stromal region of the cornea) by scraping with Bard-Parker blade #15. Buprenorphine 0.02 mg/kg was given as postoperative analgesia (IM). In order to evaluate the healing mechanism of a PCED condition, and since steroids have the ability to delay healing, all groups were dosed with Dexamethasone (DEX). Recombinant human growth hormone (rHGH) was chosen as a positive control; rHGH has been shown to accelerate wound healing. BSS served as a negative control. All animals started treatment on day 1 post-surgery. Each test group was dosed topically with DEX (50 µL) QID within 8 hours. The DEX+rHGH (n=5) and DEX+BSS (n=4) groups were dosed topically QID (50 µL) at least 30 minutes after the DEX treatment. One group consisted of the DEX alone treated group (n=4). Clinical ophthalmic examinations, including slit lamp biomicroscopy and fluorescein staining, were performed twice daily on days 2, 3, 4, and once on day 5. Percentage of corneal wound healing was evaluated and compared across the treatment groups using Image J software. The groups were compared statistically using ANOVA and LSD multiple comparison procedure (GraphPad software, San Diego, CA) and histopathologically.

Results
Day 2 through day 5, the DEX group was inhibited in the corneal wound healing rate (Table 1, Figure 1). DEX+BSS and DEX+rHGH were similar on day 2. By day 3 to day 4 the DEX+rHGH was significantly faster in corneal wound healing rate when compared to the DEX+BSS and the DEX alone treated group (Figure 1 and 2). By day 4 PM and day 5 both DEX+rHGH and DEX+BSS were similar but still significantly different in healing rate when compared to the DEX alone group. Mild to moderate conjunctival congestion was persistent in all groups during day 1 and 2; in addition, mild iris injection of the secondary vessels was observed. By day 3, these ocular conditions began to dissipate with the ocular scores slightly less in the DEX+rHGH and the DEX+BSS groups. Day 4 the ocular observations were normal except for the corneal wound area.

Conclusion
This model can offer the opportunity for evaluating pharmacological agents and drug delivery systems that can promote corneal wound healing for PCED conditions.