Re-epithelialization in Cornea Organ Culture After Chemical Burns and Excimer Laser Treatment

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Objective: To describe the epithelial healing rates observed in freshly cultured rabbit corneas chemically burned with high-concentration hydrochloric acid (HCl) and sodium hydroxide (NaOH) and subsequently treated with phototherapeutic keratectomy (PTK).

Methods: We obtained 126 fresh corneoscleral rims from cadaveric New Zealand white rabbits. Each cornea was exposed to 4-mm cellulose sponges soaked in a solution of topical 0.9% isotonic sodium chloride solution, 2M HCl, or 0.5M NaOH. A transepithelial PTK (6-mm zone; 100-µm ablation depth) was then performed using the excimer laser (150-mJ/cm² energy pulse; 20 nanosecond duration; and 10-Hz frequency). Corneas were placed in tissue culture, and 1 cornea from each group was taken out of culture each day after treatment. Re-epithelialization was monitored by means of fluorescein staining, slitlamp photography, and histopathological analysis.

Results: Corneas treated with HCl and NaOH exhibited immediate epithelial defects that slowly healed over time. In PTK-treated corneas, the re-epithelialization rate was accelerated compared with that of controls (P = .003 for the HCl group, and P < .001 for the NaOH group). The new epithelial layers were smoother in PTK-treated corneas, as confirmed by results of histopathological analysis.

Conclusion: Corneal damage caused by HCl and NaOH may be modulated in vitro by PTK in this rabbit model.

Clinical Relevance: After corneal chemical damage, 193-nm excimer laser PTK accelerates epithelial wound healing.

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MATERIALS AND METHODS

RABBIT GLOBE HARVEST

We followed the guidelines of the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Vision and Ophthalmic Research and approval from the Institutional Animal Care and Use Committee of the University of California–Irvine to enucleate a total of 162 eyes from cadaveric New Zealand white rabbit heads within 8 hours of death. Globes were maintained in a cold and moist chamber until use.

TITRATION EXPERIMENTS

In preliminary trials, concentrations and exposure times required for superficial corneal damage of up to 150 μm in depth were determined for both chemicals. Thirty-six rabbit corneas were exposed to 4-mm disks of methylcellulose surgical sponges (Visitec Inc, Sarasota, Fla) soaked in increasing concentrations of NaOH (0.025M-0.5M) or HCl (0.3M-2M). The disks were applied to the central cornea for variable exposure times of 5, 8, and 10 seconds. The chemically exposed corneas were then rinsed copiously with balanced salt solution and fixed for histological analysis.

CORNEAL CHEMICAL BURNS

The globes selected for the study (n=126) were assigned to the following 3 different groups of 14 eyes each: (1) HCl group, (2) NaOH group, and (3) control group. Each group was then subdivided into non–PTK-(n=7) and PTK-treated corneas (n=7). The experiments were performed in triplicate. The presence of epithelial defects, corneal scars, or signs of ocular-corneal inflammation were used as exclusion criteria.

Corneas were exposed to a solution of 2M HCl for 10 seconds or 0.5M NaOH for 5 seconds to induce chemical burns, or to 0.9% isotonic sodium chloride solution as a control. Immediately after exposure, all globes were washed 3 times in a 100-mL bath of 0.9% isotonic sodium chloride solution for 5 minutes to remove chemical residues. Corneas and a 2- to 3-mm scleral skirt were excised using curved corneal scissors. Any attached iris and ciliary body were gently removed without damaging the endothelium.

PREPARATION OF WHOLE-ORGAN RABBIT CORNEA CULTURE

With the use of a jigsaw, the ends of laboratory test tubes (2099; Falcon, Oxnard, Calif) were cut at the indicator line nearest the bottom. The cut edges of the domes were smoothed, and the domes were autoclaved in a screw-cap jar. After sterilization, the cut edges of the domes were coated with autoclaved high-vacuum grease (Dow-Corning, Midland, Mich) and aseptically placed firmly concave-side down into each well of a 12-well tissue culture plate. Dulbecco Modified Eagle Medium (Gibco BRL, Life Technologies Inc, Rockville, Md) supplemented with 10% fetal bovine serum was then added to each well to just cover each dome (Figure 1).

EXCIMER LASER PTK OF CHEMICALLY BURNED CORNEAS

A clinical excimer laser system (VISX Star S4; VISX Inc, Santa Clara, Calif) was used to perform transepithelial PTK ablations on 7 corneas from each group, immediately after rinsing. Laser settings of 150 mJ/cm², 20 nanosecond pulse duration, and 10-Hz repetition rate were used with a 6-mm ablation zone. Four hundred sixteen pulses were applied to the selected corneas to remove approximately 100 μm of anterior corneal tissue.

Ablated and nonablated corneas were pooled and washed 3 times with Dulbecco Modified Eagle Medium supplemented with penicillin G sodium, streptomycin sulfate, and amphotericin B. We used sterile forceps, grasping only the scleral rims and not the cornea, to place each cornea over a single dome with culture medium in the previously prepared well plates. Incubations were performed in 7.5% carbon dioxide and 92.5% air at 37°C for the duration of the experiments, with culture medium changed every 2 days.

MONITORING OF EPITHELIAL WOUND HEALING

Each day after treatment, 1 PTK- and 1 non–PTK-treated cornea from each group were removed from culture. Each removed cornea was placed in a tissue well plate provided with domes as a support but devoid of medium, and was stained with 1% sodium fluorescein solution. The plate was vertically fixed in a slitlamp, where cobalt blue photography at 16× original magnification was performed (Nikon FS-3; Nikon Corp, Torrance, Calif). Thereafter, corneas were fixed in 10% buffered formaldehyde for histopathological analysis.

The obtained 35-mm slides from the slitlamp photography were digitally scanned at a resolution of 1280×960 pixels (Scan Maker 35T; Microtek, Compton, Calif). The images were subsequently processed with digital imaging software (Scion Image; Scion Corp, Frederick, Md) to quantitatively assess the fluorescein staining area corresponding to the epithelial defect.

STATISTICAL ANALYSIS

Numerical data were analyzed using commercially available software (SYSTAT 9.0 for Windows; SPSS Inc, Chicago, Ill). Epithelial defect area from each group per day and percent-age of change from baseline were described using mean and SD. Because of small numbers, defect areas between the PTK- and non–PTK-treated subgroups were compared using non-parametric tests (Mann-Whitney rank sum test). A P value of <.05 or less was considered statistically significant.


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to ablate the damaged tissue, mean re-epithelialization was almost complete at day 4 of the observation period (Table). The change was more evident in the NaOH group, showing significant differences in epithelial defect area at every day of the study when comparing PTK with non–PTK-treated corneas. That is, PTK accelerated the rate of epithelial wound closure in both types of chemical burn (Figure 2). In both cases, PTK resulted in closure of a wound that remained open without such treatment during the experiments (Figure 3).

Light microscopy revealed that epithelial cells seemed to migrate more readily over the laser-ablated stroma, as compared with the non–PTK-treated specimens in the HCl and NaOH groups. Remnants of necrotic tissue appeared to block the cell repopulation of the epithelial defect, especially in the NaOH group. Although a normal appearance of the rabbit epithelial cell layers was not achieved in this organ culture system, a multilayered aspect resembling a normal epithelium was more likely to be observed in the laser-ablated corneas (Figure 4). The HCl group displayed a hypocellular stroma during the 3

Figure 1. A, Schematic of the culture system in a lateral view. The end of the test tube is placed upside down. B, Overview of the culture system with corneas in place. The top right well of the plate has no cornea to show the dome.

Figure 2. Phototherapeutic keratectomy (PTK) curve shows a progressive and faster epithelial defect closure pattern over time. In the non–PTK-treated corneas, epithelial closure is more erratic in the hydrochloric acid (HCl) group (A) compared with the sodium hydroxide (NaOH) group (B). However, the NaOH group showed reversal of the healing process at day 3.

| Percentage of Change per Day in Epithelial Defect Area in Groups of Study* |
|---------------------------------|-----------------|--------|-----------------|-----------------|--------|
|                                | HCl             | HCl + PTK | P Value         | NaOH            | NaOH + PTK | P Value |
| Day                             |                 |          |                 |                 |          |        |
| 1                               | 29.6            | 63.6     | .0495           | -14.7           | 74.4      | .0495  |
| 2                               | 26.7            | 78.2     | .13             | 70.3            | 91.2      | .0495  |
| 3                               | 3.9             | 87.8     | .0495           | 72.5            | 97.2      | .0495  |
| 4                               | 77.0            | 85.6     | .83             | 38.6            | 98.1      | .0495  |
| Overall                         | 34.3            | 78.8     | .003            | 41.7            | 90.2      | <.001  |

*Percentage of change is compared with baseline. Negative value indicates a larger epithelial defect than baseline. Unless otherwise indicated, data are given as percentages. HCl indicates hydrochloric acid; PTK, phototherapeutic keratectomy; and NaOH, sodium hydroxide. Chemical burns are described in the “Titration Experiments” subsection of the “Materials and Methods” section.
days after the exposure, but after this period, a normal appearance in keratocyte cell density was again observed.

Several organ culture models have been proposed to study the corneal in vitro wound-healing process.4-8 Air/liquid organ cultures seem to improve the epithelial cell morphology while decreasing the intercellular edema usually observed in conventional submerged models.9-11 With this in mind, we have developed a simple model that allows corneal re-epithelialization after excimer laser-induced injury.3 Our goal was to adopt these models to evaluate the effects of corneal chemical burns in the re-epithelialization process in vitro. Furthermore, we sought to evaluate the potential therapeutic benefits of PTK after such chemical exposures. Survival time is an important limiting factor with this culture system, since corneas remained intact for only 5 days. After the sixth day in culture, signs of system failure were seen.

The healing of rabbit corneal alkali wounds in vitro has previously been investigated in a whole-organ culture model.12 In that study, a 5.5-mm disk of filter paper soaked with a 1N NaOH solution was applied for 60 seconds to the central cornea, and then the cornea was copiously rinsed and placed in culture. Re-epithelialization of the wound was complete in 5 days at 37°C incubation. We expected to accelerate this healing process by laser-ablative removal of chemically damaged tissue from the central corneal region of our whole-organ cornea culture injury model. Almost complete epithelial defect closure was achieved after 3 days in culture in the PTK-treated corneas, in contrast to larger defects and delayed re-epithelialization rates in the non-PTK-treated corneas. The PTK-treated corneas

Figure 3. Day 4. A, Hydrochloric acid (HCl)–induced burn with irregular surface and epithelial defect. B, HCl-induced burn followed by PTK shows complete re-epithelialization and a smooth surface. C, Sodium hydroxide (NaOH)–induced burn with central necrotic tissue (arrows). D, NaOH-induced burn followed by PTK shows smooth surface without defects (original magnification ×16).
showed constant and progressive re-epithelialization rates over time, whereas the non–PTK-treated corneas exhibited more erratic patterns. In the non–PTK-treated corneas, the epithelial defect was found to be enlarged at days 3 (in the HCl group) and 4 (in the NaOH group), showing a regression of the healing process. In addition, the epithelial defect increased the first day after NaOH exposure, indicating a persistent, deleterious effect of the chemical even 24 hours after exposure.

In this study, we have demonstrated that, in a controlled ex vivo tissue culture system, the excimer laser may be used to remove damaged central corneal tissue shortly after exposure to strong acidic and basic solutions. This process might even promote a faster re-epithelialization process. However, we cannot confidently state that these results would mimic the in vivo situation. In corneal scrape wounds, a 3-day epithelial repair has been observed in live animal studies. Despite clinical evidence that PTK may be helpful to promote epithelial healing in patients with recurrent erosions late after a chemical injury, a similar effect has not been documented, to our knowledge, in the immediate acute phase after a chemical burn. Using the same corneal damage variables determined in this study, experiments are under way to reproduce these results in a live rabbit model. It is our belief that PTK after chemical burns of the cornea may be beneficial to limit the extension of the corneal damage and to promote a more adequate substrate for epithelial migration. In the clinical situation, we would advocate initial manual removal of loose necrotic tissue followed by PTK removal of remaining chemically damaged stroma. In our study, remnants of necrotic tissue appeared to further impede epithelial healing. Removing this dead tissue should enhance the subsequent PTK effect. Confirmatory experiments are currently in progress.

Excimer laser corneal ablation is now such a widespread technique that this method could become widely available. However, the main difficulty lies in the judgment of the necessary depth of ablation. We are currently characterizing the spectral properties of the cornea during excimer laser ablation of normal and chemically damaged corneas in hopes of defining an optical indicator of the transition between normal and diseased tissue. Pulse-to-pulse monitoring of corneal tissue fluorescence might allow the surgeon to determine precisely when abnormal tissue has been sufficiently ablated to accelerate the healing process. However, further in vivo work is needed to assess the potential efficacy of this method in a clinical environment. We would expect immediate PTK to be most effective. Even copious rinsing of chemically damaged tissue is unlikely to remove all of the offending chemical agent, which will further diffuse into the cornea unless mechanically removed. It remains to be seen exactly how sensitively ablative excimer laser–induced...
corneal fluorescence detects chemical and chemically damaged tissue.

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