Gelatinase B and A Expression After Laser In Situ Keratomileusis and Photorefractive Keratectomy

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Objective: To compare the expression of gelatinases in the corneal epithelium and stroma after laser in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK).

Methods: Rabbit eyes were treated with LASIK (n=11), PRK (n=12), or corneal flap construction (n=12); 4 eyes served as unwounded controls. Zymography was performed on the central epithelium and the stroma 1, 3, and 7 days after surgery to determine the expression of gelatinases.

Results: Epithelial expression of gelatinase B in the LASIK group (0%-25%) was lower than that in the PRK group (50%-100%) and was identical to the corneal flap group. Stromal expression of gelatinases A and B was similar after LASIK and PRK, but was minimal after corneal flap construction at all time points. Epithelial expression of gelatinase A was similar for the first 3 days after LASIK and PRK but not thereafter.

Conclusions: Gelatinase B epithelial expression was up-regulated after PRK but not after LASIK. Gelatinase B stromal expression was up-regulated after both procedures.

Clinical Relevance: Differences in wound healing and subepithelial scarring after these 2 procedures may be related to gelatinase B.


Excimer laser photorefractive keratectomy (PRK) and laser in situ keratomileusis (LASIK) are used to modify corneal shape to correct refractive errors. Compared with LASIK, PRK more often results in corneal subepithelial haze leading to reduced best-corrected visual acuity.1,3 Gelatinases A and B are important members of the matrix metalloproteinase family, consisting of protein-cleaving enzymes that degrade extracellular matrix and basement membrane components.6-7 Matrix metalloproteinases may play a role in corneal wound healing and scarring after PRK.8-10

In the rabbit cornea, gelatinase B expression peaks 1 to 3 days after surgery in the epithelium and returns to baseline 7 to 10 days after surgery. Gelatinase A is expressed in the keratocytes of unwounded corneas and peaks 1 to 3 days after PRK.9 Construction of a corneal flap before PRK during LASIK preserves the structural integrity of the epithelial basement membrane zone and seems to reduce postoperative corneal haze.3

We have reported increased corneal light scattering as seen by scatterometry in rabbit eyes treated with –10 diopters (D) PRK compared with –10 D LASIK.5 This study tests the hypothesis that the higher incidence of corneal haze after PRK than after LASIK may be associated with greater expression of matrix metalloproteinases.

RESULTS

After PRK, central corneal epithelial defects were closed by days 3 to 7. A faint reticular haze was observed 7 days after PRK. In eyes that were treated with LASIK or in which corneal flaps were constructed, an arcuate epithelial defect was seen at the edge of the flap on day 1 that healed by day 3. There was no evidence of corneal epithelial ingrowth, infection, ulceration, or haze after LASIK or flap surgery.

Gelatinases B and A migrated on the gels as 92- and 72-kd bands of gelatin digestion, respectively. The epithelial expression of gelatinase B in the LASIK group (0%-25%) was similar to that in the PRK group at all time points (50%-100%) and was identical to the corneal flap group. Stromal expression of gelatinases A and B was similar after LASIK and PRK, but was minimal after corneal flap construction at all time points. Epithelial expression of gelatinase A was similar for the first 3 days after LASIK and PRK but not thereafter.

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MATERIALS AND METHODS

Thirty-nine eyes from pigmented rabbits were divided into 4 groups: Group 1 (n=11) received LASIK treatment. Group 2 (n=12) received PRK treatment. Group 3 (n=12) received corneal flaps and no laser treatment. Group 4 (n=4) was the control group and was not treated. Rabbits were anesthetized with an intramuscular injection of a 1:1 mixture of ketamine hydrochloride (40 mg/kg body weight) and xylazine hydrochloride (7 mg/kg body weight). Preparacaine hydrochloride and atropine sulfate eye drops were used for topical anesthesia and cyclopia. The surgical procedures were performed as described earlier.1 In the LASIK group (group 1), with a rotating microkeratome we constructed a nasally based corneal flap approximately 100 µm thick and 8 mm in diameter. We then performed a 5-mm, −10 D PRK ablation (89-µm stromal ablation) using an excimer laser (Visx, Santa Clara, Calif), and repositioned the flap. In the PRK group (group 2), we scraped the epithelium with a No. 15 blade, and performed a 5-mm, −10 D PRK stromal ablation. In group 3, we constructed corneal flaps similar to those of group 1 and then repositioned and secured them without excimer laser treatment. All eyes received the same postprocedural medications and treatments—atropine eye drops, topical erythromycin ointment, and 24 hours of tarsorrhaphy. Rabbits were examined every 24 to 48 hours after surgery.

Rabbits in groups 1, 2, and 3 were killed 1, 3, or 7 days, respectively, after surgery with an intravenous overdose injection of pentobarbital (n=4/time point). Control rabbits (group 4) were killed on day 7. The epithelium within a 6.5-mm area demarcated by a trephine was scraped and immediately frozen at −70°C. Stromal buttons were frozen at −70°C after scraping the endothelium. Zymography was performed to determine matrix metalloproteinase expression as previously described.8,10 Briefly, 0.1% gelatin (Sigma, St Louis, Mo) was mixed with 10% acrylamide before standard sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Corneal tissue was homogenized in 100 µL of sample buffer and incubated at room temperature for 5 minutes. The supernatant (20 µL) was placed in the electrophoresis wells and later incubated with 2.5% Triton X-100 (Sigma) for 1 hour. Proteinase reactions were carried out for 18 hours at room temperature. Gels were stained with Coomassie blue, destained with a solution of 7% methanol and 7% acetic acid, dried, and photographed. HT-1080 human fibrosarcoma cell–conditioned media (American Tissue Culture Collection, Rockville, Md) was run concomitantly (positive control). The percentage of corneas showing enzyme activity (visible bands) was calculated.

Figure 1. Gelatinase B expression after photorefractive keratectomy (PRK), laser in situ keratomileusis (LASIK), or corneal flap. Left, Expression of gelatinase B in the corneal epithelium is shown as the percentage of corneas with identifiable matrix metalloproteinase–9 bands on zymography on days 1, 3, and 7 after wounding. Right, Expression of gelatinase B in the corneal stroma after wounding.

COMMENT

Corneal wound healing after PRK differs from healing after LASIK and may account for the differences in the procedure outcomes.3,5,11 After LASIK, there is minimal corneal subepithelial scarring and haze, and the visual recovery is more rapid compared with that found after PRK. In addition, the presence of a corneal flap with LASIK reduces interactions between the epithelial and stromal layers.3,5,11

We observed that there is less synthesis of gelatinases in the corneal epithelium during the first week after treatment with LASIK compared with PRK. The regenerating corneal epithelium after PRK is metabolically active, producing several proteolytic enzymes, such as gelatinases A and B.7,8,12-15 Evidence from several studies suggests that destruction of the epithelial adhesion structures in the basement membrane zone may be the primary role of gelatinase B after deep keratectomy and ulcerating corneal wounds, and this may occasionally interfere with normal reepithelialization.5,8,10,12

In this study, we observed up-regulation of gelatinase B expression in the corneal epithelium in the first week after PRK, but not after LASIK or after the construction of corneal flaps. In contrast, expression of gelatinase B in the stroma was higher after both PRK and LASIK, but was negligible after corneal flaps. The epithelial pattern for gelatinase A after LASIK was not parallel to that after the construction of corneal flaps. We did not compare these results with those after epithelial scrape wounds.
In previous studies we showed that gelatinase B synthesis is consistently induced after deep keratectomy and PRK wounds, but not after superficial epithelial scraping. We further used in situ hybridization and confocal microscopy to localize gelatinase B, and observed that it is synthesized by the basal cell layers of migrating epithelial cells, particularly at the leading edges over the PRK ablation bed. This would place the enzyme in the appropriate location to induce remodeling of the anterior stroma. In these wounds, epithelial migration occurs over the stroma in the first 3 to 4 days, prior to re-formation of the basement membrane. It is thus unlikely that gelatinase B is primarily involved in basement membrane degradation. In addition to their role in the remodeling of the anterior stroma, gelatinases A and B may play a role in inhibiting the angiogenesis that is known to occur in other systems of wound healing. Gelatinase B is involved in angiostatin production. We are investigating whether the absence of neovascularization after excimer laser keratectomy wounds is directly related to matrix metalloproteinase expression.

The mechanism of corneal scar formation after PRK is not fully understood. The expression of gelatinases A and B in the stroma in our study was essentially identical after LASIK and PRK, but gelatinase B expression was minimal after flap surgery. This is consistent with the notion that gelatinase production may be an essential component of stromal remodeling after keratectomy wounds.

There may have been quantitative differences in the production of gelatinase A that we were unable to measure, particularly because the expression of gelatinase A is constitutively present in the stroma of normal unwounded corneas (Figure 2, right). Alternatively, corneal scarring and subepithelial haze may be primarily related to epithelial rather than stromal factors, including gelatinase B production. Additional studies are needed to understand the exact mechanisms of scar formation after PRK, which may lead to a new target for therapeutic strategies.

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REFERENCES


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