Tear Fluid Plasmin Activity After Excimer Laser Photorefractive Keratectomy

Timo Tervo,* Tuula Virtanen,* Niina Honkanen,† Matti Härkönen,† and Ahti Tarkkanen*

Purpose. Elevated tear fluid plasmin activity may correlate with delayed healing of corneal wounds. The present study was performed to establish the tear fluid plasmin activity after photorefractive keratoablation (PRK).

Methods. Tear fluid aspirated with microcapillaries was subjected to a fluorometric plasmin assay using the 7-amido-4-trifluoromethylcoumarin derivate of the tripeptide H-D-Val-Leu-Lys as substrate.

Results. Tear fluid flow, plasmin activity, and flow-corrected plasmin excretion rate in tears (plasmin flux) were determined preoperatively and 1, 2, and 7 days after PRK. The preoperative tear fluid flow was 6.55 /xl/min (median; range, 1.8 to 21.8 /xl/min), plasmin activity was 1.29 IU/l (median; range, 0.6 to 6.9 IU/l), and the excretion of plasmin in tears was 11.7 /xlU/min (median; range, 1.6 to 41.5 /xlU). A statistically significant decrease in tear fluid plasmin activity was found during the follow-up period on the first (0.6 IU/l; range, 0.6 to 1.7 IU/l, P < 0.01) and second (0.65 IU/l; range, 0.6 to 1.49 IU/l, P < 0.01) postoperative days. On the other hand, significant elevation of both tear fluid flow and plasmin flux values occurred during the first two postoperative days. The median plasmin flux values on days 1, 2, and 7 were 57.35 /xlU/mm² (range, 16 to 540 /xlU/min, P < 0.01), 40.0 /xlU/min (range, 13.3 to 222.8 /xlU/min, P < 0.01), and 10.2 /xlU/min (range, 2.2 to 90.7 /xlU/min, P > 0.05), respectively.

Conclusion. The marked elevation of tear fluid flow coincided with the persistence of an epithelial defect. However, because of the acceleration of tear fluid flow, proteolytic activity due to plasmin (IU/l) actually decreases. Consequently, the increased excretion of plasmin in tears (plasmin flux) does not lead to highly elevated plasmin activity, which could inhibit wound healing. It seems to be a natural healing response because all corneas were epithelialized normally by or on day 3. Invest Ophthalmol Vis Sci. 1994; 35:3045-3050.

Postoperative haze and regression of the achieved refractive result are major problems in excimer laser photorefractive keratectomy (PRK) of the cornea.¹² Corticosteroids have been used in preventing haze,⁵–⁶ but their effect is controversial⁶ and they may cause side effects such as elevation of the intraocular pressure and cataract formation. Basically, the action of corticosteroids is inhibitory to the reparative stage of wound healing, that is, synthesis and rearrangement of new extracellular matrix and restoration of cellular components of the stroma and epithelium.⁴–⁷ However, if the first stage of wound healing, debridement or “cleaning” of the wound from damaged tissue components is overactive, healing of the lesion may be delayed.⁸ Proteolytic mechanisms are thought to be important both for organization and remodeling of normal tissues⁸ and for regulation of wound healing.⁸–¹⁰ Plasminogen-plasmin system,⁸–¹⁰ matrix metalloproteinases,⁹¹¹ mast cell proteinases,¹² leukocyte-mediated mechanisms,¹³ and activation of the complement system are examples of such enzyme systems.

Healing of experimental keratectomy wounds is associated with a short-lasting elevation of tear fluid proteolytic activity due to plasmin.¹⁴ However, if ex-
cessive proteolytic activity lasts a long period of time, it may disturb corneal healing. In an attempt to find ways of preventing scarring and development of haze after PRK, we have monitored the changes in tear fluid flow and plasmin activity during healing of PRK wounds.

MATERIALS AND METHODS

Patient Group

This group included 16 patients with myopia being treated for PRK. An FDA-applied informed consent form was signed by each patient before the operation. Each patient volunteered to give tear fluid samples during routine check-up visits. The study protocol was accepted by the ethical committee of Helsinki University Eye Hospital and it followed the tenets of Declaration of Helsinki.

Preoperative Investigations

Preoperative investigations included determination of refraction, corneal topography, keratometry, measurement of the axial length, Schirmer test in topical anesthesia with eyes closed, and routine slit-lamp examination.

Control Group

Preoperative tear fluid samples from all 16 patients with PRK were examined for tear fluid flow, plasmin activity, and volume-corrected excretion of plasmin. Similarly, six additional control samples (manipulated tear fluid-samples) were obtained 5 to 10 minutes after measurement of the axial length of the eye in topical anesthesia.

Excimer Laser Photorefractive Keratectomy

All ablations were performed using a VisX 20/20 (VisX, Sunnyvale, CA) excimer laser. The spherical equivalent of the corrections varied from 3 to 10 D, with diameters of 6 mm. A single 6-mm PRK was performed if the correction was less than 6 D, and corrections between 6 to 10 D were performed using a two-zone program.

Postoperative Medication

Postoperative medication consisted of oral, nonsteroidal anti-inflammatory drugs for 3 to 4 days, eye patch, and chloramphenicol ointment for 2 to 3 days, followed by a topical antibiotic–corticosteroid combination (Maxitrol, Alcon, Fort Worth, TX) 2 to 3 times a day, 4 to 12 weeks.

Tear Fluid Plasmin Assay

Tear fluid (10- to 80-μl) samples were collected with calibrated 5- or 25-μl blunted microcapillaries. The collection time was measured by calculating how long it took to fill the scaled microcapillaries for estimation of the tear fluid flow. Samples were centrifuged immediately in Eppendorf (Merck, Darmstadt, Germany) tubes at 8900g for 1 minute. Supernatants were aspirated and plasmin activity was measured immediately, or the samples were frozen on dry ice and stored at −75°C until they were assayed. The tear fluid proteolytic activities were measured using a fluorometric plasmin assay described previously. The assay is based on the use of a lyophilized substrate kit containing the 7-amido-4-trifluoromethylcoumarin derivative of the tripeptide H-D-Val-Leu-Lys. The final substrate concentration in the assay was 1 mmol/l and the buffer medium was 50 mmol/l Tris-HCl, pH 8.0, containing 0.1% bovine serum albumin (BSA). Tris(hydroxymethyl)aminomethane (T-1378) and BSA (A-7638) were obtained from Sigma (St. Louis, MO). The World Health Organization human plasmin standard from the National Institute for Biological Standards and Control (London, UK) was used as a plasmin calibrator in the assay. The reaction was initiated by adding either 5 to 10 μl of plasmin standard (10 IU/l) or the sample into the reaction medium. The activity was determined as a two-point assay with a Transcon 102 FN fluoro-nephelometer (Elomit, Helsinki, Finland) at room temperature. After the initial fluorescence, the final fluorescence was measured after 5 to 10 minutes. Plasmin activity in the sample was calculated from the fluorescence intensity change compared to the calibrator. The detection limit of the plasmin activity assay is 0.6 IU/l.

Statistical Analysis

Wilcoxon’s signed-rank test (the two-group paired test) was used to examine the significance of the incidence of the tear fluid flow, plasmin activity, and plasmin flux of the follow-up group at different times after the operation.

RESULTS

Preoperative plasmin activity of the patients with PRK (N = 16) was 1.29 IU/l (median, range 0.6 to 6.9 IU/l). Tear fluid flow in the collection capillary was 6.55 μl/min (median, range 1.8 to 21.8 μl/min) and the corrected excretion of plasmin (plasmin flux) 11.7 μIU/min (median, range 1.6 to 41.5 μIU/min) (Fig. 1).

One day after the excimer laser surgery, all operated eyes were tearing and there was an epithelial defect of 3 to 4 mm in diameter. Tearing of the eyes resulted in a 15-fold elevated tear fluid flow (95.6 μl/min; range 18.4 to 900 μl/min), leading to relatively low plasmin activity (0.6 IU/l; range 0.6 to 1.7 IU/l). However, the median plasmin flux was increased by
Tear Fluid Plasmin After PRK

FIGURE 1. Medians of plasmin activity, tear-fluid flow, and plasmin flux of excimer laser photorefractive keratoablation patients (N = 16) before and 1, 2, and 7 days after surgery. Postoperative values (days 1, 2, and 7) were compared with preoperative values. **P < 0.01. Ranges are given in the Results section.

The factor of five (57.35 μIU/min; range 16 to 540 μIU/min). The changes in the flow and plasmin flux were statistically significant, as was the decrease in plasmin activity levels (Fig. 1).

On the second postoperative day, the eyes continued to show considerable conjunctival and some limbal hyperemia. There was still a small (approximately 1 to 2 mm wide) epithelial defect. Both tear fluid flow (56.8 μl/min; range, 12.1 to 300 μl/min) and plasmin flux (40.0 μIU/min; range, 13.3 to 228.8 μIU/min) had decreased but were significantly higher than the preoperative values. The median plasmin activity (0.65 IU/l; range, 0.6 to 1.5 IU/l) was higher than on the first preoperative day but was still significantly lower than the preoperative activity (Fig. 1).

Only three eyes showed a minimal epithelial defect on day 3. All eyes were epithelialized on day 4.

On day 7, the median values of plasmin activity (1.15 IU/l; range, 0.6 to 11.3 IU/l), tear fluid flow (6.7 μl/min; range, 2.7 to 15 μl/min) and plasmin flux (10.2 μIU/min; range, 2.2 to 90.7 μIU/min) values had returned to preoperative levels (Fig. 1). All the eyes now appeared clinically normal, and there were no epithelial defects.

Manipulated tears (preoperative tear-fluid samples studied 5 minutes after measurement of the axial length in topical anesthesia) showed higher plasmin activity (2.3 IU/l; range, 0.6 to 8.5 IU/l), yielding after correction with the tear fluid flow (7.5 μl/min; range, 1.5 to 20.0 μl/min) a plasmin flux of 16.6 μIU/min (range, 4.9 to 66.9 μIU/min). The plasmin flux was significantly higher than that obtained without manipulation (P = 0.01).

DISCUSSION

Low plasmin activity10,16,17,18,19,20 as well as activators of plasminogen8,20,21 are detectable in normal human tear fluid. A low tear-fluid secretion rate seems to be associated with somewhat higher plasmin activity in healthy eyes of control individuals.18 However, if the plasmin activity data were corrected with the tear-fluid flow rate, there was no difference in excretion of plasmin.18 The results of this study also show that tear-fluid flow, which increased noticeably on the first two days after surgery, is an important factor and affects the concentration of tear-fluid components. The increase in tear-fluid secretion necessitated the use of 25-μl microcapillaries instead of the 5-μl capillaries, which were too small to suck all the fluid secreted. The parameter “tear-fluid flow” in the collection capillary is used in this study. It is not identical with production of tears per unit time because in our method the residual fluid in conjunctival fornix is also aspirated. Moreover, some stimulation of reflex tearing may occur during collection of tears in spite of gentle aspiration technique. We can assume that the preoperative plasmin activity (IU/l) should have been a little higher than was measured here because of induction of some reflex tearing. On the other hand, the overflow of tears on the first two days after surgery made it easy to collect tears without further stimulation of reflex tearing, but some of the tears might have been missed. This would mean that the changes shown to take place after PRK—that is, decrease in plasmin activity but increase in plasmin excretion (plasmin flux)—should have
been even more pronounced than was actually revealed in this study. Although a rather gross estimate, the capillary tube method seems to be the simplest way to have reasonable volume correction in determination of tear fluid components. This is, to our understanding, necessary in studies like this because of the clinically evident wide variation in tear fluid production after corneal wounding. Consequently, comparison of tear fluid parameters determined in separate studies may not be easy, especially if tear fluid flow rate has not been taken into account.

The present study clearly shows an excess of plasmin excretion in tears lasting 2 to 3 days. We originally also determined the immediate postoperative plasmin activity. However, due to the presence of damaged cells and tissue components as well as to use of topical wetting agents and antibiotics during the operation, these results were too variable. The results shown here are generally similar to our previous experimental keratomeotomy wound study on rabbits. Unlike in some other studies, plasmin activity was lower rather than elevated during the first 1 to 2 postoperative days. Several factors seem to contribute to this difference. Tear fluid of an eye recently subjected to PRK undoubtedly contains both epithelial and stromal cells, extracellular matrix components, and inflammatory cells. Centrifugation eliminates the cell-bound plasmin or plasminogen activators in loosened epithelial, stromal, or inflammatory cells. Furthermore, the fluorometric method is more specific for plasmin activity than the caseinolytic procedure. The elevation of plasmin excretion was mainly due to a significant acceleration of the tear-fluid flow rate, whereas the plasmin activity decreased. This elevation was parallel with the persistence of an epithelial defect and might also correlate with the surface area of the epithelial defect or with the length of the advancing wound edge. After healing of the epithelium (day 7), the parameters measured (tear fluid flow, plasmin activity, and flux) were at the levels of the preoperative ones.

Notably, our plasmin assay measures only active plasmin. Consequently, inhibitor-bound inactive plasmin molecules are not detected. Moreover, enzyme activities present in tears do not necessarily completely reflect the intracorneal state and the balance between proteinases and tissue-repairing enzymes. For such purposes, histochemical methods may be used.

When wound healing is concerned, plasmin activity is probably a more important factor than plasmin flux. The present results showed that plasmin activity decreases after PRK because of a remarkable elevation of tear fluid flow. Consequently, reflex tearing seems to be important for wound healing. It decreases the concentration of proteolytic enzymes, eliminates tissue debris, and provides the wound with increased amounts of tear fluid-derived growth factors. We have recently proposed that recognition and management of dry eye conditions by punctual occlusion may reduce development of haze after PRK.

As concluded before, some plasmin activity is probably needed for normal healing of epithelia. It would be produced as a result of activation of plasminogen to active plasmin by the cells of the leading edge. However, long-lasting and high plasmin activity seems to inhibit epithelial healing. Normal human tear fluid also contains both plasminogen activators and plasmin inhibitors. Although some plasmin activity is probably generated during the normal re-epithelialization process, high plasmin activity can also result from the inflammatory process, presence of leukocytes, microbial activators or consumption or degradation of naturally occurring plasmin inhibitors in tear fluid. The present study also shows that the normal relationship between plasmin activity and tear fluid flow returns as soon as re-epithelialization is completed. As indicated by the results obtained after measurement of axial length, activation of plasminogen may take place even after nontraumatic manipulation of the eye.

Published studies show that corneal tissue after wounding remains in the reconstruction phase an unusually long time, up to 1 year. During this period the cornea is synthesizing new collagen, hyalurionate, adhesion complexes, and ground substance components, as well as fibronectin and tenascin. Furthermore, locally synthesized or tear-fluid derived growth factors may modulate healing rate.

The PRK-induced neural damage might also inhibit the healing process and tear secretion. The epithelial endings, basal epithelial plexus, and anterior stromal nerve trunks are cut during the operation. Deep ablations may cause almost complete destruction of the stromal nerve trunks located in the anterior third of the stroma. Corneal sensitivity is usually restored in about 2 months, but anatomic recovery may never be complete. Inhibition of wound healing by sensory denervation seems to be more evident in rabbits than in humans. Consequently, sensory disturbances might contribute to the healing problems associated with corrections over 10 D because of phenomena resembling neuroparalytic keratitis. Patients also often report a decrease of reflex tearing during the first 2 months after surgery when corneal sensitivity is still decreased.

Key Words
excimer laser, tear fluid, human, plasmin activity, fluorometric assay

References
1. Lohman C, Gartry D, Kerr Muir M, Timberlake G, Fitzke F, Marshall J. 'Haze' in photorefractive kera-
Tear Fluid Plasmin After PRK

1. Pfister RR, Haddox JL, Dodson RW, Deshazo WF.
2. Butrus SI, Ochsner KI, Abelson MB, Schwartz LB.


35. Tuft SJ, Rawe JM. Photorefractive keratectomy: Im-


37. Tervo K, Pääläysaho T, Latvala T, Tervo T. Effect of excimer-laser refractive surgery on corneal innerva-

38. Beuerman RW, Schimmelpfennig B. Sensory denerva-